PB80-220080

Dioxins. Volume II. Analytical Method for Industrial Wastes

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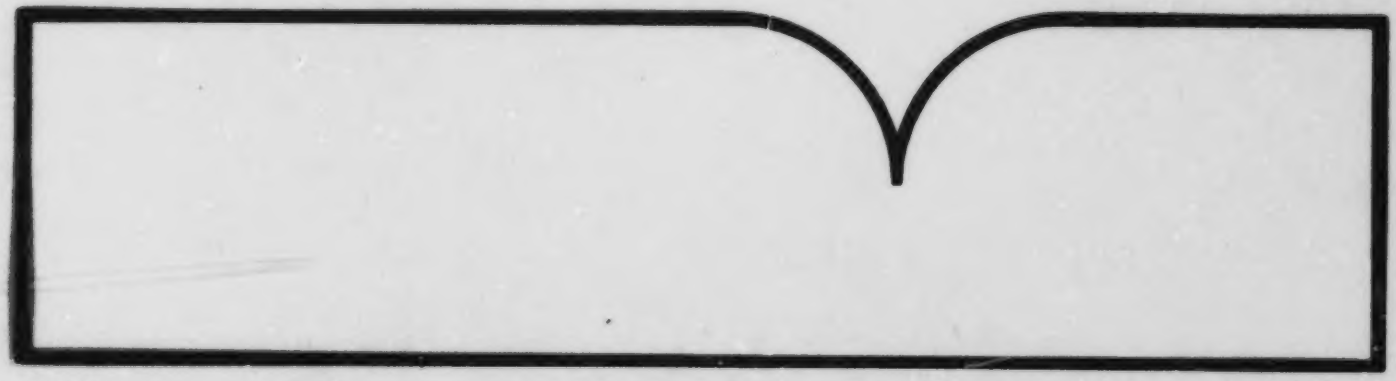
Prepared for

Industrial Environmental Research Lab.
Cincinnati, OH

Jun 80



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EPA-600/2-80-157
June 1980

Research and Development

PB80-220080

EPA

Dioxins

Volume II.
Analytical Method for
Industrial Wastes

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-80-157	2.
3. RECIPIENT'S ACCESSION NO. PB80 220080	
4. TITLE AND SUBTITLE Dioxins: Volume II. Analytical Method For Industrial Wastes	5. REPORT DATE JUNE 1980 ISSUING DATE.
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) T. O. Tiernan, M. L. Taylor, S. D. Erk, J. G. Solch, G. Van Ness, and J. Dryden	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS The Brehm Laboratories and Department of Chemistry Wright State University, Dayton, Ohio 45435	10. PROGRAM ELEMENT NO. 1BB610
	11. CONTRACT/GRANT NO. Contract No. 68-03-2659
12. SPONSORING AGENCY NAME AND ADDRESS Industrial Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268	13. TYPE OF REPORT AND PERIOD COVERED Final, 10/78 to 3/79
	14. SPONSORING AGENCY CODE EPA/600/12

15. SUPPLEMENTARY NOTES

Volume II of a three-volume series on dioxins

16. ABSTRACT

The overall objective of this research project was to develop a unified analytical approach for use in quantifying ppt levels of tetrachlorodibenzo-p-dioxins (TCDD's) in various chemical wastes. Waste samples from plants manufacturing trichlorophenol, pentachlorophenol, and hexachlorophene, and from plants processing wood preservatives were provided by the EPA.

The extraction procedure developed for isolating the TCDD's from the various types of sample matrices is fully described. Analysis was accomplished using highly specific and sensitive coupled gas chromatographic-mass spectrometric (GC-MS) methods. Both low and high resolution MS techniques were employed. This methodology is also described in detail. The procedures presented in this report were acceptable for most of the industrial process samples provided. TCDD's were detected and quantitatively determined in several of the samples at levels in the ppt to ppm range. One sample, identified as a trichlorophenol stillbottom, was found to contain 40 ppm TCDD's. This method was not applicable for wood or woodlike products and difficulties were also encountered with some samples that were susceptible to emulsion formation in the preparation stages.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Organic chemicals Pesticides Chemical analysis Industrial wastes	Dioxins; 2,3,7,8-TCDD Analytical chemistry Hazardous waste disposal	07C 07D 13B

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

☆U.S. GOVERNMENT PRINTING OFFICE: 1980--657-165/0008

EPA-600/2-80-157
June 1980

DIOXINS:
VOLUME II.
ANALYTICAL METHOD FOR INDUSTRIAL WASTES

by

T. O. Tiernan, M. L. Taylor, S. D. Erk,
J. G. Solch, G. Van Ness, and J. Dryden
The Brehm Laboratory and Department of Chemistry
Wright State University
Dayton, Ohio 45435

Contract No. 68-03-2659

Project Officer

David R. Watkins
Industrial Pollution Control Division
Industrial Environmental Research Laboratory
Cincinnati, Ohio 45268

INDUSTRIAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

When energy and material resources are extracted, processed, converted, and used, the related pollutional impacts on our environment and even on our health often require that new and increasingly more efficient pollution control methods be used. The Industrial Environmental Research Laboratory-Cincinnati (IERL-Ci) assists in developing and demonstrating new and improved methodologies that will meet these needs both efficiently and economically.

This report is one of a three-volume series dealing with a group of hazardous chemical compounds known as dioxins. The extreme toxicity of one of these chemicals, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), has been a concern of both scientific researchers and the public for many years. The sheer mass of published information that has resulted from this concern has created difficulties in assessing the overall scope of the dioxin problem. In this report series the voluminous data on 2,3,7,8-TCDD and other dioxins are summarized and assembled in a manner that allows comparison of related observations from many sources; thus, the series serves as a comprehensive guide in evaluation of the environmental hazards of dioxins.

Volume I is a state-of-the-art review of dioxin literature. Detailed information is presented on the chemistry, sources, degradation, transport, disposal, and health effects of dioxins. Accounts of public and occupational exposure to dioxins are also included. Volume II details the development of a new analytical method for detecting part-per-trillion levels of dioxins in industrial wastes. It also includes a review of the analytical literature on methods of detecting dioxins in various types of environmental samples. Volume III identifies various routes of formation of dioxins in addition to the classical route of the hydrolysis of chlorophenols. The possible presence of dioxins in basic organic chemicals and pesticides is addressed, and production locations for these materials are identified.

For further information, contact Project Officer David R. Watkins, Organic and Inorganic Chemicals Branch, IERL-Ci. Phone (513) 684-4481.

David G. Stephan
Director
Industrial Environmental Research Laboratory
Cincinnati

PREFACE

This report is Volume II in a series of three reports dealing with a group of hazardous chemical compounds known as dioxins. This volume discusses the development of a new analytical technique for identifying dioxins in industrial wastes, and presents a bibliography of other analytical methods for determining dioxins in various types of environmental samples. Other volumes of this series examine the occurrence, environmental transport, toxicity, and disposal of this class of compounds, the detailed chemistry of dioxin formation, and the commercial products with potential for containing dioxin contaminants.

An extensive body of published literature has appeared during the past 25 years that has been concerned primarily with one extremely toxic member of this class of compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Often described in both popular and technical literature as "TCDD" or simply "dioxin," this compound is one of the most toxic substances known to science. This report series is concerned not only with this compound, but also with all of its chemical relatives that contain the dioxin nucleus. Throughout these reports, the term "TCDD's" is used to indicate the family of 22 tetrachlorodibenzo-p-dioxin isomers, whereas the term "dioxin" is used to indicate any compound with the basic dioxin nucleus. The most toxic isomer among those that have been assessed is specifically designated as "2,3,7,8-TCDD."

The objective in the use of these terms is to clarify a point of technical confusion that has occasionally hindered comparison of information from various sources. In particular, early laboratory analyses often reported the presence of "TCDD," which may have been the most-toxic 2,3,7,8-isomer or may have been a mixture of several of the tetrachloro isomers, some of which are relatively nontoxic. Throughout this report series, the specific term 2,3,7,8-TCDD is used when it was the intent of the investigator to refer to this most-toxic isomer. Since early analytical methods could not dependably isolate specific isomers from environmental samples, the generic term "TCDD's" is used when this term appears to be most appropriate in light of present technology.

ABSTRACT

The overall objective of this research project was to develop a unified analytical approach for use in quantifying part-per-trillion levels of tetrachlorodibenzo-p-dioxins (TCDD's) in various chemical wastes.

The EPA provided Brehm Laboratory of Wright State University with 17 waste samples from plants manufacturing trichlorophenol, pentachlorophenol, and hexachlorophene, and from plants processing wood preservatives.

The extraction procedure developed for isolating the TCDD's from the various types of sample matrices is fully described. Analysis was accomplished using highly specific and sensitive coupled gas chromatographic-mass spectrometric (GC-MS) methods. Both low and high resolution MS techniques were employed. This methodology is also described in detail. The procedures presented in this report were acceptable for most of the industrial process samples provided. TCDD's were detected and quantitatively determined in several of the samples at levels in the ppt to ppm range. One sample, identified as a trichlorophenol stillbottom, was found to contain 40 ppm TCDD's. This method was not applicable for wood or woodlike products and difficulties were also encountered with some samples that were susceptible to emulsion formation in the preparation stages.

The Brehm Laboratory submitted this report in fulfillment of a subcontractual effort with Battelle Columbus Laboratories, supported through a prime contract between Battelle and the U.S. Environmental Protection Agency (Contract No. 68-03-2659). This report covers the period October 1, 1978 to March 31, 1979 and work was completed as of March 3, 1979.

CONTENTS

	Page
Foreword	iii
Preface	iv
Abstract	v
Figures	viii
Tables	ix
List of Abbreviations	x
Acknowledgment	xi
1. Introduction	1
2. Analytical Background	4
3. Analytical Method	8
4. Discussion and Results	13
5. Conclusions and Recommendations	43
References	45
Appendix A. Principles of GC-MS	49
Appendix B. Other Instrumental Methods	57
Appendix C. Literature Review	60

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FIGURES

Number		Page
1	Mass chromatogram of extract of EPA sample 2 at m/e 322 obtained with GC-QMS.	16
2	High pressure liquid chromatogram of sample 2.	23
3	High pressure liquid chromatogram of 2,3,7,8-TCDD standard.	24
4	Four-ion mass fragmentogram of benzene solvent blank obtained with GC-MS-30.	26
5	Four-ion mass fragmentogram of 50 pg of 2,3,7,8-TCDD and 1 ng $^{37}Cl_4$-2,3,7,8-TCDD obtained with GC-MS-30.	27
6	Four-ion mass fragmentogram of sample 12700 obtained with GC-MS-30.	28
7	Four-ion mass fragmentogram of sample 5 obtained with GC-MS-30.	29
8	Dual-ion mass fragmentogram of sample 2, obtained with GC-MS-30, mass resolution 1:12,500.	35
9	Dual-ion mass fragmentogram of 150 pg of 2,3,7,8-TCDD standard obtained with GC-MS-30, mass resolution 1:12,500.	36
10	Mass fragmentograms using GC-MS-30 of mixtures of 2,3,7,8-TCDD with other chlorinated compounds.	38
11	Mass spectrum of 2,3,7,8-TCDD standard and sample 2 (mass range m/e 330 to m/e 250).	41
12	Mass spectrum of 2,3,7,8-TCDD standard and sample 2 (mass range m/e 250 to m/e 150).	42

TABLES

Number		Page
1	Samples Used in Development of Analytical Method for TCDD's in Industrial Wastes	14
2	Elution of TCDD's in Extracts of Sample 2	17
3	Content of TCDD's in Column Fractions for Sample 2	19
4	Elution of TCDD's in Extracts of Sample CO4131	20
5	Results of GC-MS-30 Analyses of EPA Samples for TCDD's	25
6	TCDD Isomer Content of Column Fractions of 2,3,7,8-TCDD Spiked Samples	31
7	Recoveries of 2,3,7,8-TCDD Spiked Samples Following Alumina Column Chromatography	33
8	Results of GC-MS-30 Analyses of $^{37}Cl_4$-2,3,7,8-TCDD Spiked Samples	34
9	Relative Intensities of Major Ions Observed in Mass Spectral Scans	40

LIST OF ABBREVIATIONS

cm	centimeter
DDE	2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene
GC-EC	gas chromatography-electron capture
eV	electron volt
g	gram
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GC-MS-30	gas chromatography-mass spectrometry (high resolution)
GC-QMS	gas chromatography-quadrupole mass spectrometry (low resolution)
HPLC	high-pressure liquid chromatography
I.D.	inside diameter
kg	kilogram
LD_{50}	lethal dose to 50% of test group
m	meter
m/e	mass to charge ratio
ml	milliliter
ml/min	milliliter/minute
mm	millimeter
MS	mass spectrometry
ng	nanogram
PCP	pentachlorophenol
PCB	polychlorinated biphenyl
pg	picogram
ppb	parts per billion (µg/l or ng/ml)
ppm	parts per million (mg/l or µg/ml)
ppt	parts per trillion (ng/l or pg/ml)
PSIG	pounds per square inch gage
2,4,5-TCP	2,4,5-trichlorophenol
TCDD's	tetrachlorinated dibenzo-p-dioxins; 22 possible isomers
µg	microgram
UV	ultraviolet
V	volt

ACKNOWLEDGMENT

This report was prepared for the U.S. Environmental Protection Agency by the Brehm Laboratory and Chemistry Department of Wright State University, Dayton, Ohio. Dr. T.O. Tiernan was the Principal Investigator with Dr. M.L. Taylor and S.D. Erk as Co-Principal Investigators. Mr. Dave Watkins was the Project Officer for the U.S. Environmental Protection Agency.

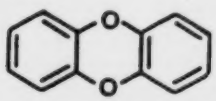
A review of the analytical literature for determination of TCDD's in various sample matrices was compiled by Battelle Columbus Laboratories, Columbus, Ohio, and constitutes a significant addition to this report. This material was used to develop Appendices B and C.

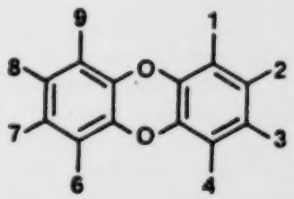
Final compilation of this report for integration into the three-volume dioxin series was done by PEDCo Environmental, Inc., Cincinnati, Ohio, with Ms. M. Pat Esposito as Project Manager. Technical assistance was provided by Ms. Diane N. Albrinck.

SECTION 1

INTRODUCTION

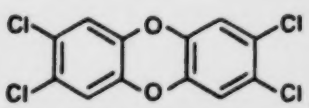
A dioxin is any of a family of compounds known chemically as dibenzo-para-dioxins. Each of these compounds has as a nucleus a triple-ring structure consisting of two benzene rings interconnected to each other through a pair of oxygen atoms. Shown below are the structural formula of the dioxin nucleus and also the abbreviated structural convention used throughout the report series.





Most environmental interest in dioxins and most studies of this family of compounds have centered on chlorinated dioxins, in which the chlorine atom occupies one or more of the eight substitution positions (Blair 1973; Lee et al. 1973; Nicholson and Moore 1979).

The interest of health and environmental researchers in chlorodioxins arose principally because of the toxicity and distribution of one of these compounds, 2,3,7,8 tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD),* whose structural formula is as follows:



* Throughout this report, the 2,3,7,8-tetrachloro isomer is specifically noted as 2,3,7,8-TCDD to differentiate it from the other tetrachloro isomers. In many cases, however, general reference is made to the family of tetra isomers as TCDD's because of the difficulty in isolating specific isomers. Refer to preface for further explanation.

This is an unusual organic chemical, symmetrical across both horizontal and vertical axes. It is remarkable for its lack of reactive functional groups and its chemical stability. It is a lipophylic molecule, virtually insoluble in water and only sparingly soluble in most organic liquids; it is a colorless crystalline solid at room temperature.

Although 2,3,7,8-TCDD was first reported in the chemical literature in 1872, no major investigations into its toxicity were begun until the 1950's. Because of the remarkable stability of this substance in biological systems and its extreme toxicity, cumulative effects of extremely small doses are a major concern. For example, the LD_{50}* of 2,3,7,8-TCDD for the male guinea pig has been shown to be only 0.6 µg/kg or 0.6 part per billion body weight (McConnell et al., 1978). Fetal mortality has been observed in rats that had been fed 10 consecutive doses of 2,3,7,8-TCDD at the level of 0.125 µg/kg per day (World Health Organization 1977). It is reasonable to presume, therefore, that the slightest trace of 2,3,7,8-TCDD in the environment may have adverse effects on the health of both human and animal populations.

In view of these considerations, it is vitally important to scrutinize carefully the probable avenues of contamination of the environment with 2,3,7,8-TCDD. It has been recognized for some time that 2,3,7,8-TCDD can be produced in the manufacture of 2,4,5-trichlorophenol. Other dioxins are similarly produced in the manufacture of other chlorophenols. The amounts of dioxins produced depend on process controls such as temperature and pressure. Since dioxins may be present in these and other manufactured chemical products, it is also likely that they may be present in the chemical wastes and sludges remaining from these processes. If this is the case, indiscriminate discharge of these wastes into the environment, or the use of improper disposal procedures could lead to the contamination of water, air, or foodstuffs. This might, in turn, result in widespread exposure of the population to TCDD's and other dioxins.

Since 1972 the personnel of the Brehm Laboratory of Wright State University have been performing sensitive dioxin analyses under programs supported by several government agencies (U.S. Air Force, U.S. Environmental Protection Agency (EPA), U.S. Department of Agriculture), and the states of Michigan, New York, and Arkansas. In these investigations Brehm Laboratory has developed and applied complex analytical methodology for the determination of TCDD's in many types of samples, including herbicides, industrial chemicals, soils, water, air, biological tissues and fluids (both human and other animal), and combustion products and related samples (Taylor et al. 1973; Taylor, Hughes, and Tiernan 1974a,b,c; Fee et al. 1975; Hughes et al. 1975; Taylor, Tiernan, and Hughes 1974; Tiernan 1975a,b; Tiernan, Taylor, and Hughes 1975; Taylor et al. 1975, 1976, 1977, 1979; Tiernan et al. 1979; Erk, Taylor, and Tiernan 1979; Yelton, Taylor, and Tiernan 1977; Wright State University 1976). The levels of TCDD's in these samples have ranged from high parts per million (ppm) to low parts per trillion (ppt). A significant number of samples examined have been found to contain

* LD_{50}: The administered dose of a substance which is lethal to 50 percent of a test group of animals.

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* LD_{50}: The administered dose of a substance which is lethal to 50 percent of a test group of animals.

detectable amounts of TCDD's. On the basis of these findings many investigators believe that TCDD's may already be widespread contaminants in the environment.

The analytical techniques applied by Brehm Laboratory in these earlier dioxin programs have varied widely in terms of the complexity of equipment, sample preparation, and the overall sensitivity and specificity of the procedures. It is now apparent that a single basic technique, amenable to minor modifications, would be desirable for the purpose of characterizing various types of chemical samples, provided that such a technique could satisfy all the specified criteria for sensitivity, specificity, and other analytical factors.

Sensitivity in the ppt range is required because of the potent toxicity of 2,3,7,8-TCDD. The current detection capability is approaching 1 ppt in at least some sample matrices and must be developed in others, particularly chemical process wastes and sludges. Accuracy is also important in these determinations, owing to current and potential regulatory actions that hinge on the analytical data.

The Brehm Laboratory, in a subcontractual effort with Battelle Columbus Laboratories, supported through a prime contract between Battelle and the U.S. EPA, has undertaken development of new analytical techniques for use in quantitating ppt levels of TCDD's in various chemical wastes. The goal in this work was to develop a unified analytical approach to the handling of a variety of chemical waste sample types and matrices.

The U.S. EPA supplied 17 test samples representing various types of chemical wastes or residues generated during the manufacture of chlorophenols and related chemicals. These samples were expected to contain TCDD's and were used in methods development by the Brehm Laboratory analysts. Presented herein are the final results of this work. This volume includes a background discussion of various analytical approaches to the detection of TCDD's, the newly developed and validated analytical method, a description of the procedures used in development of the method, and the analytical data obtained in applying the method to various industrial samples. Appendix A of this report discusses general principals of gas chromatography and mass spectrometry. Appendix B discusses other methods and procedures found in current literature which may be used to detect TCDD's in a variety of sample matrices. Appendix C is a compilation of references on analysis of TCDD's, categorized by sample type.

SECTION 2

ANALYTICAL BACKGROUND*

Analytical methods for detecting TCDD's in various types of samples involve extensive sample preparation procedures followed by highly complex instrumental analysis. This section discusses various approaches to the detection and quantitative measurement of TCDD's, which had been used prior to the inception of the present study in 1978.

SAMPLE PREPARATION

Because TCDD's may be found in a variety of matrices many different sample extraction/preparation methods have been developed. Although they differ in complexity, most of these methods may be classified into two major categories: first, those characterized by a highly basic extraction step, and second, those involving only neutral extraction. The neutral extraction technique was developed to preclude the possibility that treatment with a strong base might generate compounds that could form chlorinated dioxins in the mass spectrometer. Following extraction, the sample preparation steps are similar for both techniques, differing only in the method of application and complexity. Both extraction procedures are described in detail below.

Basic Extraction Method

Historically basic extraction methods were first developed for the determination of TCDD's in environmental samples (Crummett and Stehl 1973; Baughman and Meselson 1973a; Baughman and Meselson 1973b). Such sample preparation techniques begin with digestion of a sample aliquot using alcohol and a strong base. This is followed by a series of organic solvent extractions to separate the TCDD's from the alkaline mixture. Solvents such as ethanol, hexane, petroleum ether, and methylene chloride have been used, either singly or in combination. The solvent extracts are combined and then subjected to a series of washings with distilled water and strong acid. The washed extract is then treated to remove all traces of water and passed through one or more chromatographic columns for removal of some co-extractants, primarily polar compounds. Instrumental analysis follows.

* Supplementary information on analytical methods for detecting dioxins in various types of samples may be found in the appendices.

An example of a typical basic extraction/preparation technique for nonfat tissue consists of heating 10 g of sample with 10 ml of ethanol and 20 ml of 40 percent potassium hydroxide solution for 30 minutes. After the solution cools, an additional 10 ml of ethanol is added and the solution is extracted with four 10-ml portions of hexane. The preparation procedure consists of washing the combined hexane extracts with concentrated sulfuric acid until the acid fraction becomes only slightly colored. The acid wash is followed by a 10 ml water wash, followed by evaporation to dryness at room temperature with a stream of dry air. The sample is then redissolved in hexane and further purified by elution chromatography using sorbents such as alumina, silica gel, or Florisil, either singly or in combination. The final eluate is concentrated prior to analysis.

Neutral Extraction Method

The neutral extraction and preparation technique was originally developed by O'Keefe, Meselson, and Baughman (1978). Albro and Corbett (1977) describe an alternative neutral extraction method. A typical neutral extraction technique for analysis of TCDD's consists of extracting the sample with 10 ml of hexane. The hexane solution is then chromatographed with a magnesia-Celite 545 column, an alumina column, an alumina minicolumn, and finally a Florisil minicolumn. The Florisil column is eluted with methylene chloride, and the eluate is concentrated in preparation for analysis. It has been asserted that neutral extraction methods are particularly effective for fish tissues and human milk (O'Keefe, Meselson, and Baughman 1978; Harless and Dupuy 1979).

Chemical Composition of Extracts

The sample preparation techniques described above are useful for destroying the integrity of the sample matrix and yield a small volume of organically miscible/soluble residue. The net effect of these clean-up procedures is the enrichment of the TCDD's relative to the natural components of the sample matrix, as well as other chlorinated environmental contaminants such as PCB's and DDE.* The latter compounds are often present in the sample in significantly greater concentrations than the TCDD's (larger by a factor of 10^6) and, therefore, may not be completely removed from the extract at this point. In addition, it is unlikely that the foregoing procedures result in separation of 2,3,7,8-TCDD from its other 21 TCDD isomers which may have been present in the sample.**

---

* DDE, or 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene, is commonly found in environmental samples; it is a degradation product of the pesticide DDT.

** Subsequent to the completion of the work described herein, reports have appeared in the literature which describe methods for synthesis and isolation of the 22 TCDD isomers (Nestrick 1979; Dow 1980). Using such new analytical procedures it is now possible to isolate and quantitatively determine 2,3,7,8-TCDD in environmental samples even in the presence of the other 21 isomers.

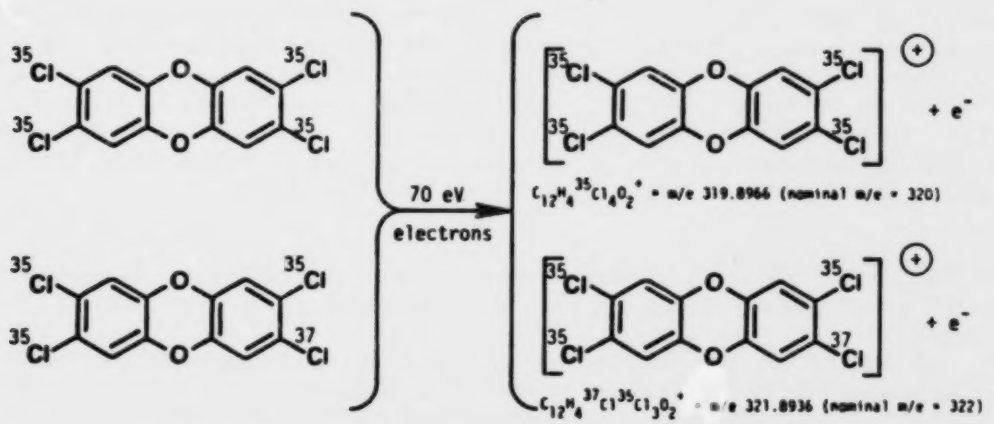
Consequently, detection and quantitation of TCDD's in general and 2,3,7,8-TCDD in particular in this "enriched" but still rather chemically complex extract can only be accomplished by using a highly specific and sensitive instrumental method. The method of choice, and that described below, is coupled gas chromatography-mass spectrometry.

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC METHODS OF ANALYSIS*

Because of its ready availability and relative ease of application, gas chromatography has been extensively used for the detection and quantitation of TCDD's (Elvidge 1971; Williams and Blanchfield 1971; Firestone et al. 1972; Williams and Blanchfield 1972; Crummett and Stehl 1973; Edmunds, Lee, and Nickels 1973; Webber and Box 1973; Buser 1976; Bertoni et al. 1978). In many instances, the authors cited above have found that the chromatographic methods lack the required specificity for determining TCDD's in complex samples. Consequently these researchers and others have sought more sensitive and specific methods of detection.

At present the analytical method which is almost exclusively used for the detection and quantitation of TCDD's is coupled gas chromatography-mass spectrometry or GC-MS (Crummett and Stehl 1973; Tiernan et al. 1975; Taylor et al. 1975; Buser and Bosshardt 1976; Harless 1976; Buser 1977; Gross 1978).

GC-MS is the only known method that can provide very high sensitivity as well as the required selectivity for TCDD's. A particularly sensitive and specific GC-MS technique which has been used entails low-resolution selective ion monitoring. In the case of TCDD's, fragment ions at nominal m/e 320 and m/e 322, as shown below, are monitored.



* A discussion of the principles of gas chromatography and mass spectrometry is presented in the Appendix.

The intensities of these ions are recorded as the TCDD's elute from the gas chromatograph. The ratio of the intensities of m/e 320 to m/e 322 is a characteristic indicator of TCDD's. Unfortunately other compounds which may also be present in the sample extract can also give rise to mass spectral ions at the same nominal masses (m/e 320 and m/e 322) as TCDD's. Two approaches can minimize this problem.

The first approach utilizes high resolution mass spectrometry ($M/\Delta M$ >9000) to increase the selectivity. The ions appearing under low-resolution MS conditions at nominal mass 322 may be produced from TCDD's which have $C_{12}H_4Cl_4O_2$ as their elemental composition and thus have an "exact" mass of 321.8936. Interfering ions such as pentachlorinated biphenyls may also appear at nominal mass 322, but their elemental composition is $C_{12}H_3Cl_5$, and therefore they have an "exact" mass of 321.8677. Thus, using high-resolution MS these ions of slightly different mass are distinguishable, and so the dioxin component having the exact mass of 321.8936 can be reliably measured. Conceivably, ions having the $C_{12}H_4Cl_4O_2$ composition can be produced from other compounds, but proper selection of chromatographic procedures maximizes the possibility of separating such compounds from TCDD's. The achievement of detection limits in the low-ppt range at high MS resolution generally requires the use of data acquisition methods which entail signal averaging (Shadoff and Hummel 1978; Gross 1978; Taylor et al. 1976).

A second approach to the problem of separating TCDD's from closely related interferences makes use of low-resolution mass spectrometry but incorporates a more selective separation step prior to the mass spectrometric analysis. Capillary column gas chromatography is useful for this purpose (Buser 1977), but liquid chromatography followed by capillary column gas chromatography has proved even more fruitful (Nestrick, Lamparski, and Stehl 1979; Dow 1980).

In both the GC-high-resolution and the GC-low-resolution mass spectrometric methods, internal standards are frequently used for the quantification of TCDD's. The analytical method developed in the present study utilizes an internal standard, namely $^{37}Cl_4$-2,3,7,8-TCDD.

SECTION 3

ANALYTICAL METHOD*

The analytical procedure ultimately developed and described herein for determination of TCDD's in various industrial process waste samples utilizes two separate GC-MS systems. A gas chromatograph coupled to a low-resolution quadrupole mass spectrometer (GC-QMS) is used for preliminary identification of TCDD's in the extracts of the waste samples. A second apparatus coupling a gas chromatograph and a high-resolution mass spectrometer (GC-MS-30) is used to confirm the results obtained with the GC-QMS technique. The analysis method entails two steps, sample preparation and instrumental analysis, as described below. It should be emphasized that, even with the elaborate separation techniques employed here, the 2,3,7,8-TCDD isomer is still not resolved from the other TCDD isomers if these are present in the sample extracts. As a result, the quantitative data obtained here for TCDD's must be considered an upper limit rather than an absolute level for any individual TCDD isomer.

SAMPLE PREPARATION

The following procedures were developed as an approach to preparation of industrial waste samples and have been successfully applied in this study.

1. Place a 2.0 g aliquot of the sample in each of the two extraction vessels. To each aliquot, add an appropriate quantity of $^{37}Cl_4$-2,3,7,8-TCDD dissolved in "distilled-in-glass" benzene as an internal standard. Spike one of the two aliquots with an additional known quantity of authentic native 2,3,7,8-TCDD at a concentration equal to the nominal amount expected in the sample.

2. Add 30 ml "distilled-in-glass" petroleum ether to each sample and mix thoroughly.

3. Extract each organic solution with 50 ml of double-distilled water and discard the aqueous layer.

4. Extract each solution with 50 ml of 20 percent potassium hydroxide and discard the aqueous basic layer.

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* This section presents the analytical method only; discussion of development of the method follows in Section 4.

5. Extract each solution with 50 ml of double-distilled water and discard the aqueous portion.

6. Extract each solution with 50 ml of concentrated sulfuric acid and discard the aqueous acidic layer.

7. Repeat step 6 until the acid layer is nearly colorless.

8. Extract each organic solution with 50 ml of double-distilled water and discard the aqueous layer.

9. Dry each organic solution over anhydrous sodium sulfate.

10. Quantitatively transfer each organic solution to another vessel, and concentrate to a volume of approximately 1 ml by passing a stream of purified nitrogen over the surface of the liquid while applying gentle heat (50°C) to the vessel.

11. Construct a chromatography column for each sample by packing a disposable glass pipette (I.D.= 0.8 cm) with glass wool and 2.8 g of Woelm basic alumina (previously activated by maintaining it at 600°C for a minimum of 24 hours, then cooled in a dessicator for 0.5 hour prior to use).

12. Quantitatively transfer each concentrated organic solution to the top of a column.

13. Elute each column with 10 ml of 3 percent "distilled-in-glass" methylene chloride in "distilled-in-glass" hexane, and discard the entire column effluent.

14. Elute each column with 20 ml of 20 percent methylene chloride in hexane and collect the eluate in four 5-ml fractions.

15. Elute each column with 10 ml of 50 percent methylene chloride in hexane and retain the entire column eluate for analysis.

16. Elute each column with 3 ml of 50 percent methylene chloride in hexane and retain the eluate for analysis.

17. Concentrate all six fractions in benzene to an appropriate volume (usually 0.1 to 1.0 ml) and proceed with analysis.

INSTRUMENTAL ANALYSIS

The application of GC-MS instrumentation methods for analysis of TCDD's requires knowledgeable and experienced personnel, dedication of the equipment, and significant capital and operating costs. The requirement for detecting low ppt levels of TCDD's in these analyses necessitates such a sensitive and selective analytical method. Because this is currently the

only known method which meets these criteria, the relatively high expense is unavoidable.

The following is a brief description of the instrumentation required for the analytical procedures developed herein.

GC-QMS System

The GC-QMS system consists of a Varian Model 2740 Gas Chromatograph coupled directly (no helium separator is required) to an Extra-nuclear Quadrupole Mass Spectrometer. The GC was adapted to include a sophisticated system of remotely actuated high-temperature switching valves (Valco Co.) and Granville-Phillips molecular leak valves, so that the column effluent could be readily regulated (Tiernan et al. 1975a; Erk, Taylor, and Tiernan 1978).

With this arrangement, the total column effluent can be directed into the mass spectrometer ion source, or the effluent flow can be split, one portion going to the ion source and the other to a gas chromatographic detector, as desired. The use of a differential high-speed pumping system on the source vacuum envelope permits introduction of as much as 65 ml/min of effluent from the gas chromatograph into the mass spectrometer ion source. Admitting the total chromatograph effluent into the mass spectrometer source enhances the sensitivity of the analysis.

For purposes of instrument control and data acquisition, the GC-QMS system is coupled to an Autolab System IV Computing Integrator. Additional capacity for off-line data reduction is available with a Hewlett-Packard 2116C Minicomputer, which is programmed to accept data (punched paper tape) from the system when necessary.

GC-MS-30 System

The GC-MS-30 system used in these studies consists of a Varian 3740 Gas Chromatograph coupled through an AEI silicone membrane separator to an AEI MS-30 Double-Focusing, Double-Beam Mass Spectrometer. The mass spectrometer is equipped with a unique electrostatic analyzer scan circuit developed by Wright State University, which permits the monitoring of as many as four mass peaks, essentially simultaneously, by rapidly and sequentially stepping and switching between the masses of interest, while maintaining picogram sensitivity for TCDD's. The data are recorded by use of a Nicolet 1074 Signal Averaging Computer.

Sample Analysis

Analysis consists of three steps as described below.

1. Analyze each eluate fraction (collected in the elution chromatography separation of the sample) on the low-resolution GC-QMS, using the following operating parameters:

Varian 2740 Gas Chromatograph

Column:	2 m x 3 mm I.D. glass packed with 3 percent OV-7 on Gas Chrom Q
Carrier gas:	Helium at 65 ml/min (the total chromatographic column effluent is admitted to the mass spectrometer ion source)
Temperatures:	Injector: 255°C Column: 275°C Transfer line: 295°C

Quadrupole mass spectrometer

Ionizing voltage:	23.5 eV
Multiplier:	3200 V
Resolution:	1:350
Source envelope pressure:	1.4×10^{-4} torr
Analyzer envelope pressure:	8.0×10^{-6} torr
Masses monitored:	m/e 320, 322
Source temperature:	250°C
Analyzer temperature:	120°C

2. Confirm any samples showing positive levels of TCDD's on the low-resolution GC-QMS by analysis of the corresponding eluate fractions using high-resolution GC-MS-30 and the following operating parameters:

Varian 3740 gas chromatograph

Column:	1.8 x 2 mm I.D. coiled glass column packed with 3 percent Dexsil 300 on Supelcoport (100/120 mesh)
Carrier gas:	Helium at a flow rate of 30 ml/min
Temperatures:	Injector: 250°C Column: 240°C Transfer line: 285°C

AEI MS-30 mass spectrometer

Resolution	1:12,500
Ionizing voltage:	70 eV
Masses monitored:	m/e 319.8966, 321.8936, 325.8805, and 327.8846
Temperatures:	Membrane separator: 215°C Transfer line: 270°C Source: 250°C

3. Determine the overall recovery of the analytical procedure by measuring the amount of internal standard ($^{37}Cl_4$-2,3,7,8-TCDD) recovered.

SECTION 4

DISCUSSION AND RESULTS

For use in developing and demonstrating the analytical methodology for determination of ppt levels of TCDD's in process wastes and related materials, samples were provided that were representative of wastes from several different industrial chemical processes that might be expected to generate chlorodioxins. The samples were obtained by the U.S. EPA from plants manufacturing trichlorophenol, pentachlorophenol, and hexachlorophene, and from plants processing wood preservatives. Initially, the nature and identity of each sample were unknown to the Wright State investigators, although information was made available early in the program about two of the samples originating from trichlorophenol manufacturing processes. Subsequently, identifying data on most of the remaining samples were obtained and are summarized in Table 1.

Because still bottom samples collected at a trichlorophenol manufacturing plant were considered of major interest, a sample of this type (EPA sample 2) was selected for use in preliminary investigations.

The initial approach to analytical method development, based on the experience of Wright State personnel in chlorodioxin analysis, is outlined below.

1. If the sample is solid, dissolve a portion in an immiscible combination of aqueous and organic solvents, such as water and petroleum ether. If the sample is a liquid, extract a portion of the material with a similar water-organic solvent system. In the absence of any prior knowledge about the content of TCDD's in a given sample, the quantity to be extracted must be selected on the basis of sensitivity of the overall technique (as indicated by previous experience) and the desired limits of detection.

2. Separate the aqueous component of the sample-solvent mixture from the organic phase and discard the aqueous portion.

3. Extract the organic fraction with sequential washes of acid, water, base, water, acid, and water (in that order), and discard the washes.

4. Concentrate the remaining organic phase to near dryness and elute through an alumina column, using appropriate solvents to separate the TCDD's and other sample components.

TABLE 1. SAMPLES USED IN DEVELOPMENT OF ANALYTICAL METHOD FOR TCDD'S IN INDUSTRIAL WASTES

EPA No.	Sample type	Source and identity of sample
C04130	Liquid slurry	Givaudan: aqueous slurry of hexachlorophene
C04131	Solid	Givaudan: activated clay filter cake from hexachlorophene manufacturing
C04132	Liquid	Givaudan: ethylene dichloride recovery solution from hexachlorophene manufacturing
2	Liquid/solid	Transvaal: still bottom from trichlorophenol (TCP) manufacturing
3	Slurry	Transvaal: cooling tank bottom from TCP manufacturing
4	Slurry	Transvaal: discharge line from TCP manufacturing
5	Liquid/solid	Transvaal: sludge from TCP manufacturing
6	Liquid	Transvaal: type unknown; presumably TCP process sample
12700	Liquid/solid	Reichold Chemical: sludge from intake of settling pond, pentachlorophenol (PCP) manufacturing
12701	Liquid	Reichold Chemical: sludge from discharge of settling pond, PCP manufacturing
12702	Solid	Reichold Chemical: PCP manufacturing
11020	Liquid/solid	Baxter: retort solids residue from wood preserving
11021	Liquid	Baxter: storage tank solution from wood preserving
11022	Liquid/solid	Baxter: cooling water solids from wood preserving
11023	Solid	Baxter: treated wood from wood preserving
11024	Solid	Baxter: soil from neighborhood of wood preserving plant
11025	Solid	Baxter: sludge from wood preserving

5. Concentrate the fraction containing TCDD's and subject it to preliminary screening analysis by use of the GC-QMS system, operated in the selected-ion monitoring mode and adjusted to detect m/e 322 and m/e 320, the two most abundant peaks in the isotopic molecular ion cluster of 2,3,7,8-TCDD.

6. If the initial screening indicates a positive level of TCDD's, then the level must be confirmed and quantitated by use of the GC-MS-30 system.

This approach was used in analysis of sample 2. Subsequent modifications of this initial procedure and other observations are discussed in following subsections.

DEVELOPING SAMPLE PREPARATION TECHNIQUE

Four aliquots of sample 2 were extracted with a mixture of water and petroleum ether. The aqueous portion was discarded, and each organic fraction was washed successively with acid, water, base, water, acid, and water. The samples were then concentrated and transferred to a 2.8 g Woelm basic alumina column (length 12 cm, internal diameter 0.8 cm).

Large quantities of a white crystalline substance appeared in the column eluate. The column apparently was overloaded owing to the large quantity of this material present in the sample. This substance possibly accounted for interference in the mass chromatogram (Figure 1). Adjustments of the column chromatography procedure were therefore made in an effort to eliminate this crystalline contaminant in the fraction containing the TCDD's.

A solvent screening study was done to evaluate the solubility of the contaminant and the potential for its removal from the sample matrix. Results are as follows:

Solvent tested	Solubility of contaminant
100% methanol	Slight solubility
3% methylene chloride in hexane	Solubility slightly greater than in 100% methanol
25% carbon tetrachloride in hexane	Solubility slightly greater than in 3% methylene chloride in hexane
100% methylene chloride	Completely soluble

Next, elution characteristics of the alumina column were evaluated. Table 2 presents the solvents and the discrete fractions collected in determining the elution characteristics of the Woelm basic alumina column.

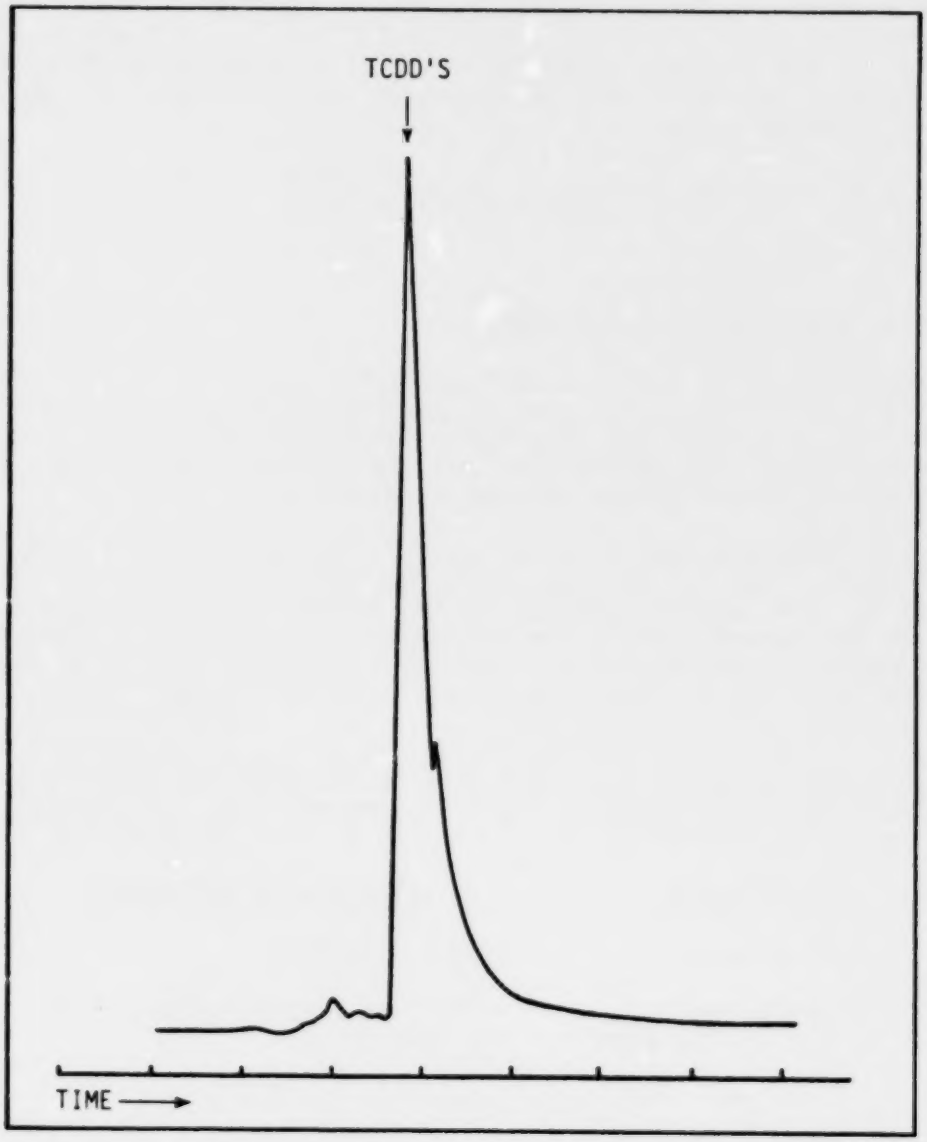

Figure 1. Mass chromatogram of extract of sample 2, at m/e 322 obtained with GC-QMS.

TABLE 2. ELUTION OF TCDD'S IN EXTRACTS OF SAMPLE 2

Set No.	Eluting solvent	Total volume of column effluent, ml	Volume of fraction(s) collected
A1	3% methylene chloride in hexane	10	total 10 ml
A2	50% methylene chloride in hexane	13	1st 5 ml in one sample; 6th through 13th ml in separate 1-ml fractions
B1	3% methylene chloride in hexane	10	total 10 ml
B2	20% methylene chloride in hexane	18	1st 5 ml in one fraction 6th through 13th ml in separate 1-ml fractions; 14th through 18th ml in one fraction
C1	25% carbon tetrachloride in hexane	10	total 10 ml
C2	50% methylene chloride in hexane	13	1st 5 ml in one fraction; 6th through 13th ml in separate 1-ml fractions
D1	25% carbon tetrachloride in hexane	10	total 10 ml
D2	20% methylene chloride in hexane	18	1st 5 ml in one fraction; 6th through 13th ml in separate 1-ml fractions; 14 through 18th ml in one fraction

Selection of the solvents and the eluate fractions was based on earlier experience of Brehm Laboratory personnel in column chromatography with similar sample matrices.

The eluate fractions were analyzed for TCDD's by use of the GC-QMS system. The results, presented in Table 3, show clearly that the best elution sequence involves the use of 10 ml of 3 percent methylene chloride in hexane, followed by 18 ml of 20 percent methylene chloride in hexane. This sequence yields TCDD's in a well-defined fraction containing few other contaminants. Use of all the other solvent pairs yielded fractions that generated interferences in the dioxin mass chromatogram which were as great as those shown in Figure 1 or greater.

Application of Initial Procedure to EPA Samples

The extraction and sample preparation procedure developed for sample 2 was applied to ten of the other industrial samples supplied by EPA. In these analyses some interferences were still present in the extract fraction which was thought to contain the TCDD's; the interferences resulted in a higher minimum detection limit (ppb) than was desired. Portions of these samples were also spiked with known quantities of 2,3,7,8-TCDD so that recoveries for the procedure could be determined. The recovery in GC-QMS analysis of sample 2 was 127 percent.

Surprisingly, in analysis of the other ten samples by the same procedure, none of the added 2,3,7,8-TCDD was recovered. The same procedure was then applied in analyses of spiked aliquots of these samples, but this time all the eluate fractions from the alumina columns were retained and analyzed for TCDD's. Again, no 2,3,7,8-TCDD was detected. It was necessary to further investigate the sample preparation procedures.

Optimizing Sample Preparation Procedure

Another sample (C04131) was subjected to the general preparation procedure already described, up to the point of elution of the column. Then the sample was spiked with a large quantity of 2,3,7,8-TCDD by introducing it directly onto the alumina column. The column elution characteristics were then evaluated as before and the results are shown in Table 4. This procedure was repeated for all other samples and their column elution profiles were determined.

This study indicated that a general extraction and preparation procedure must include a provision for assessing the elution characteristics of the alumina column for each type of sample matrix. Apparently, each type of sample conditions or deactivates the column in a manner peculiar to its matrix, and this conditioning in turn, determines the elution characteristics of TCDD's, which may differ markedly in different sample types.

TABLE 3. CONTENT OF TCDD'S IN COLUMN FRACTION FOR SAMPLE 2[a]

Solvent set No.	Eluate fraction no.[b]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	TCDD's detected																	
A1	-*	-*	-*	-*	-*	-*	-*	-*	-*	-*	0	0	0	0	0	0	0	0
A2	+*	+*	+*	+*	+*	+*	+*	+*	+	-	-	-	-	0	0	0	0	0
B1	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
B2	+*	+*	+*	+*	+*	+	+	+	+	+	+	+	+	+	+	+	+	+
C1	0	0	0	0	0	0	0	0	0	0								
C2	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	0	0	0	0	0
D1	0	0	0	0	0	0	0	0	0	0								
D2	+*	+*	+*	+*	+*	+*	+*	+	+	+	+	+	+	+	+	+	+	+

[a] Aliquots of EPA sample 2.
[b] Fraction numbers refer to those collected from each of the columns, as indicated in Table 2.

+ = TCDD's present in fraction.
- = No TCDD's detected in fraction.
0 = Fraction not analyzed.
* = Two or more peaks evident in mass chromatogram near 2,3,7,8-TCDD retention time.

TABLE 4. RECOVERY OF 2,3,7,8-TCDD SPIKE FROM ELUATES OF SAMPLE C04131

Solvent	No. of fractions collected	Volume of each fraction	Action	Results
10 ml 3% methylene chloride in hexane	1	10 ml	Discarded	
20 ml 20% methylene chloride in hexane	4	5 ml	Analyzed by GC-QMS	No 2,3,7,8-TCDD
10 ml 50% methylene chloride in hexane	1	10 ml	Analyzed by GC-QMS	80% 2,3,7,8-TCDD recovered

ANALYTICAL PROCEDURE

Research workers in several laboratories, including the Brehm Laboratory, have analyzed various types of samples for dioxin content. Generally, the analytical approach to determining a chlorinated hydrocarbon of this type in a complex sample matrix has involved quantitation of the chlorocarbon by use of electron capture-gas chromatography (EC-GC) or gas chromatography-mass spectrometry (GC-MS). The studies at Brehm Laboratory entailed use of GC-MS and high-performance liquid chromatography (HPLC).

GC-MS System

As described in Section 3, the GC-QMS system was used for initial detection of TCDD's in the fractionated sample. Then the GC-MS-30 was used to confirm the positive levels of TCDD's detected in the GC-QMS.

In one procedural modification, a labelled internal standard, $^{37}Cl_4$-2,3,7,8-TCDD, was added to all samples. Also, the MS-30 high-resolution mass spectrometer was modified to permit essentially simultaneous step-scanning of four ions in the high-resolution mode. The ions typically monitored were:

m/e 319.8966, a major molecular ion in the mass spectrum of 2,3,7,8-TCDD

m/e 321.8936, a major molecular ion in the mass spectrum of 2,3,7,8-TCDD

m/e 325.8805, a molecular ion indicative of interfering PCB's

m/e 327.8846, a major molecular ion in the mass spectrum of $^{37}Cl_4$-2,3,7,8-TCDD.

High-Performance Liquid Chromatography (HPLC)

In earlier studies aimed at determining TCDD's in environmental samples, concern has been raised that the presence of the so-called predioxins (for example, polychlorinated phenoxyphenols) in the samples would lead to false positive determinations of TCDD's because the latter can be formed by cyclization reactions of the predioxins in the hot injection port of gas chromatographs. The present investigation ruled out potential false positive effects of predioxins by applying an HPLC analytical technique as a quality assurance measure. HPLC does not entail injection of the sample into a heated port and therefore minimizes the possibility of thermal cyclization of predioxins.

The HPLC instrument used in these studies is the Model LC 5021 Varian. This microprocessor-controlled HPLC is both completely automatic and programmable and incorporates a multiple solvent system. Three detectors are available: a fixed-wavelength UV (254 nm) detector, a variable-wavelength UV detector, and a fluorescence detector. A cathode ray tube (CRT) keyboard

unit displays operating parameters while a micropressor-based computing integrator (DCS-111L) stores the data and performs appropriate calculations. The parameters applicable to the instrument as it was used in this study are listed below:

Column:	DuPont Zorbax ODS (25 cm x 6.2 mm)
Temperature:	50°C
Starting Pressure:	952 psig
Solvent:	100% Methanol
Flow rate:	2.5 ml/min
Detector:	UV (235 nm)
Sensitivity:	0.02 absorbance units full scale/15 ng TCDD's

Upon injection of a 10 µl aliquot of the sample 2 extract into the HPLC, a chromatographic peak having a retention time which was the same as that observed with the 2,3,7,8-TCDD standard was observed. Representative HPLC chromatograms are shown graphically in Figures 2 and 3, and these results indicate a readily detectable level of TCDD's in the sample 2 extract. It is apparent that the TCDD's detected cannot have been formed by cyclization of predioxins.

Analytical Results

Attempts were made to extract 15 of the 17 EPA samples by the procedures described in section 3. The remaining two samples, 11023 and 12702, were not subjected to these methods. Sample 11023 was a section of wood, which the earlier experience of Wright State had shown is not amenable to a potassium hydroxide digestion process. Sample 12702 was not analyzed because of insufficient time during the contract period.

Twelve of the fifteen samples were successfully analyzed by the Wright State procedure, with results as shown in Table 5. These data show that the procedure is applicable to samples exhibiting a wide range of concentrations of TCDD's from ppt to ppm (a factor of 10^6). For those samples in which no TCDD's were detected, the minimum detectable concentration of TCDD's was in the low ppt range (45 to 140 ppt).

Examples of mass fragmentograms obtained with the GC-MS-30 high resolution mass spectrometer are shown in the following figures. Figure 4 shows a four-ion step-scan mass fragmentogram of benzene, the solvent used for dilution of the final sample residue. Analysis of a solvent blank is repeated before analysis of each sample in order to ensure that no TCDD's are carried over in the injection syringe. Figure 5 illustrates similar data obtained from injection of a sample consisting of 50 pg of native 2,3,7,8-TCDD and 1 ng of $^{37}Cl_4$-2,3,7,8-TCDD. Note that different attenua-

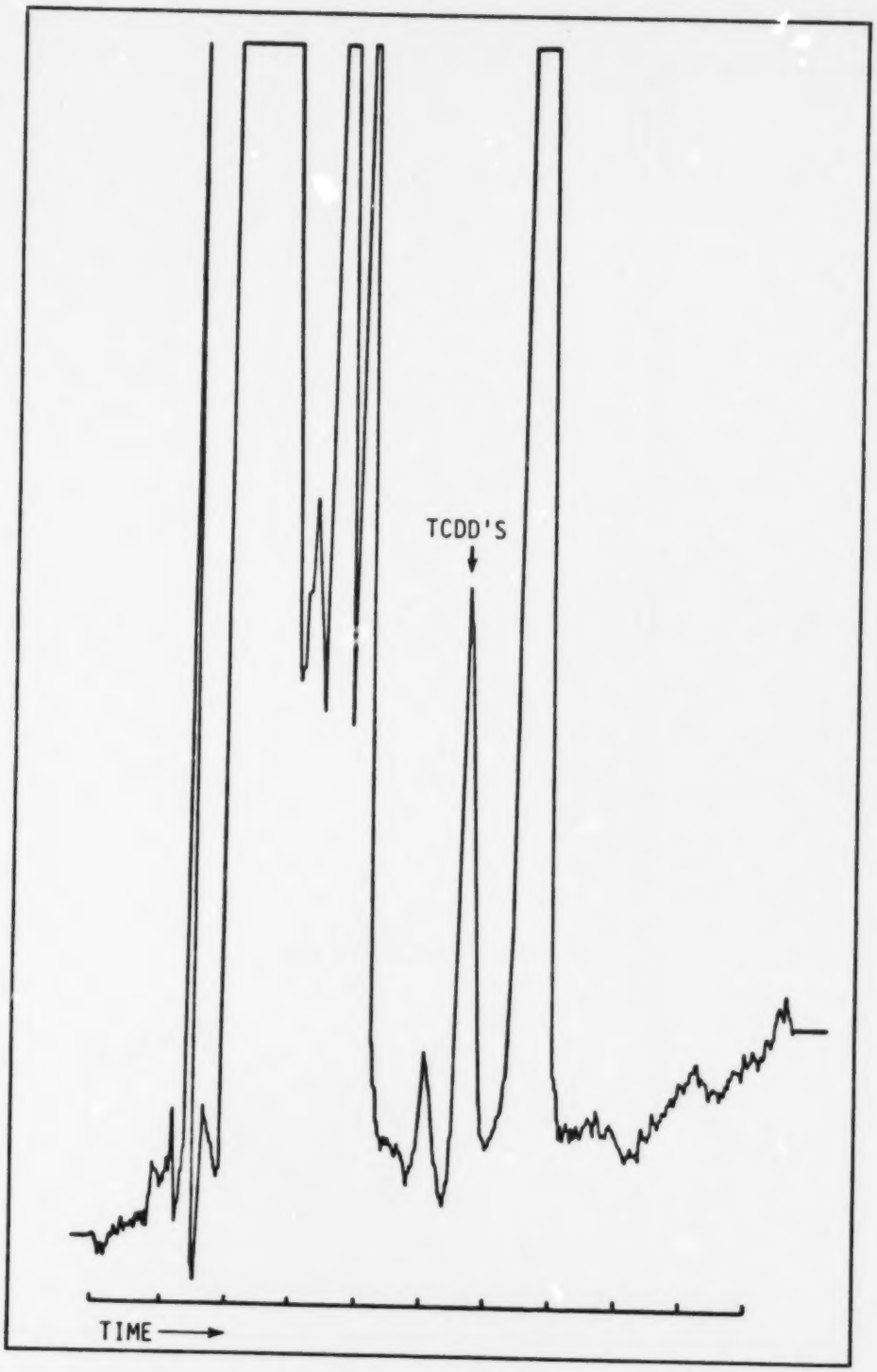

Figure 2. High pressure liquid chromatogram of sample 2.

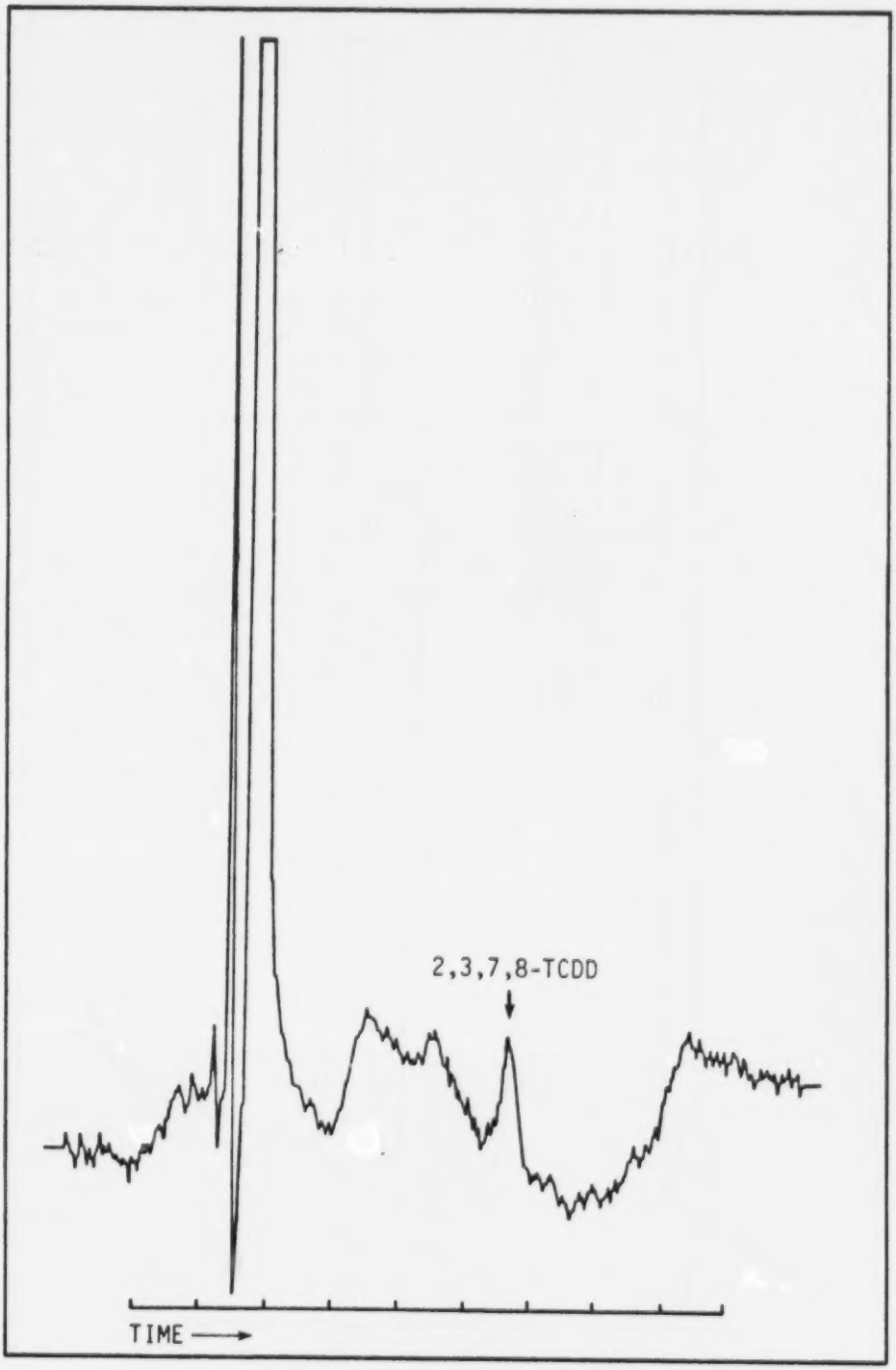

Figure 3. High pressure liquid chromatogram of 2,3,7,8-TCDD standard.

TABLE 5. RESULTS OF GC-MS-30 ANALYSIS OF EPA SAMPLES FOR TCDD'S

EPA sample no.	Origin	Quantity of TCDD's found ng/g (ppb)	Minimum detectable concentration pg/g (ppt)
C04130	Givaudan	ND[a]	140
C04131	Givaudan	ND	70
C04132	Givaudan	ND	50
2	Transvaal	40,000	e
3	Transvaal	675	e
4	Transvaal	22	e
5	Transvaal	070	e
6	Transvaal	ND	50
12700	Reichold	ND	80
12701	Reichold	ND	75
12702	Reichold	b	
11020	Baxter	ND	140
11025	Baxter	ND	45
11021	Baxter	c	
11022	Baxter	c	
11023	Baxter	b	
11024	Baxter	d	

[a] ND: no TCDD's detected in excess of the minimum detectable concentration.
[b] Not processed.
[c] General procedure could not be successfully applied to these samples.
[d] Not analyzed on GC-MS-30.
[e] An exact minimum detectable concentration was not recorded for these analyses; however the reported values for quantity of TCDD's found are well above the criterion of 2.5X noise.

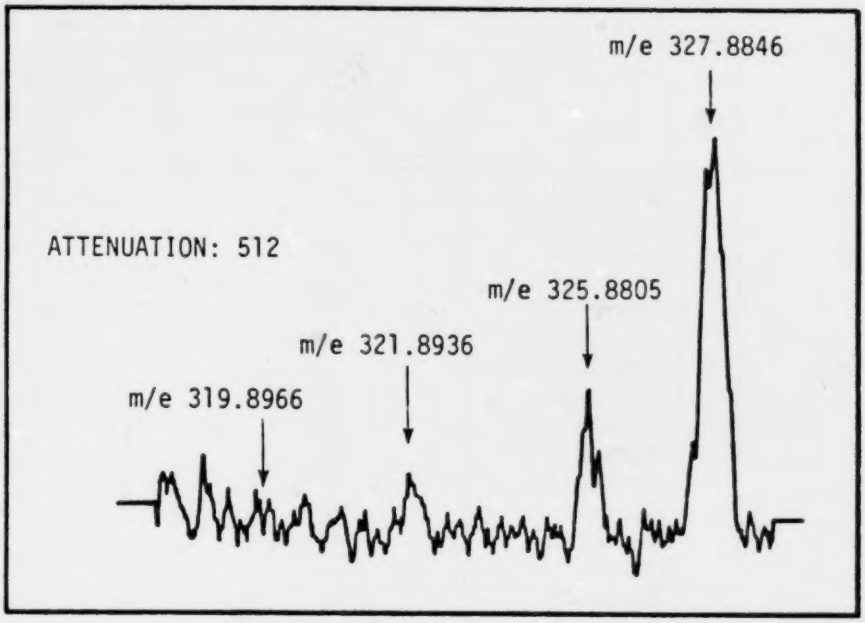

Figure 4. Four-ion mass fragmentogram of benzene solvent blank obtained with GC-MS-30.

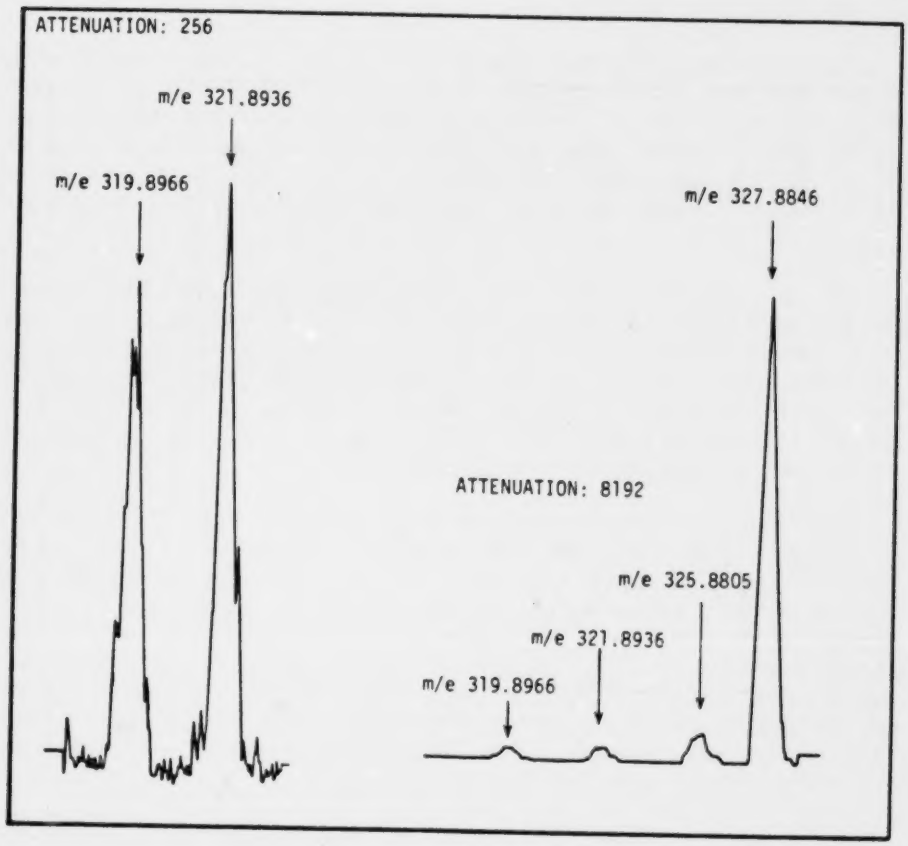

Figure 5. Four-ion mass fragmentogram of 50 pg 2,3,7,8-TCDD and 1 ng $^{37}Cl_4$-2,3,7,8-TCDD obtained with GC-MS-30.

tions have been applied to the various peaks displayed in Figure 5. Figures 6 and 7 demonstrate similar four-ion step-scan mass fragmentograms obtained for two of the EPA samples. Although the fragmentogram for sample 12700 shows peaks at m/e 319.8966 and m/e 321.8936, their intensities are not greater than 2.5 times the background; this is one of the criteria applied for establishing the presence of TCDD's in a sample. Based on the recovery of $^{37}Cl_4$-2,3,7,8-TCDD from sample 12700, the minimum detectable concentration (MDC) of TCDD's is 80 pg/g.

The mass fragmentogram for sample 5 (Figure 7) shows peaks at both m/e 319.8966 and m/e 321.8936, and the intensities are well in excess of 2.5 times the background levels. After application of a recovery correction on the basis of the internal standard, these data indicate that sample 5 contains 70 pg TCDD's per gram of sample. Data similar to those shown in Figures 4 through 7 were obtained for the other samples analyzed in this program.

Analyses of samples 11021 and 11022 were not completed owing to the formation of an intractable emulsion at the petroleum/ether interface. Analysis of sample 11024 on the GC-MS-30 system was not attempted because a colored residue was visible in the final extract. Earlier experience had shown that such residues indicate that the sample extract contains gross quantities of compounds other than TCDD's, which lead to serious contamination of the high-resolution mass spectrometer.

All data in Table 5 were derived from analyses with the high resolution GC-MS-30 system. For each of the industrial process samples, the appropriate elution chromatogram fractions to be analyzed were determined in advance in a series of alumina column elutions using an aliquot of the sample spiked with 2,3,7,8-TCDD standard; these elutions were accomplished in a manner similar to that described for sample 2. These elution test samples were analyzed with the low resolution GC-QMS system. Data pertinent to the determination of the elution characteristics of TCDD's in the various samples are shown in Table 6. The fractions collected for each sample in the elution experiments are as follows:

1. Fraction I - First 5-ml portion eluted with 20 percent methylene chloride in hexane.

2. Fraction II - Second 5-ml portion eluted with 20 percent methylene chloride in hexane.

3. Fraction III - Third 5-ml portion eluted with 20 percent methylene chloride in hexane.

4. Fraction IV - Fourth 5-ml portion eluted with 20 percent methylene chloride in hexane.

5. Fraction V - First 10-ml portion eluted with 50 percent methylene chloride in hexane.

6. Fraction VI - Last 3-ml portion eluted with 50 percent methylene chloride in hexane.

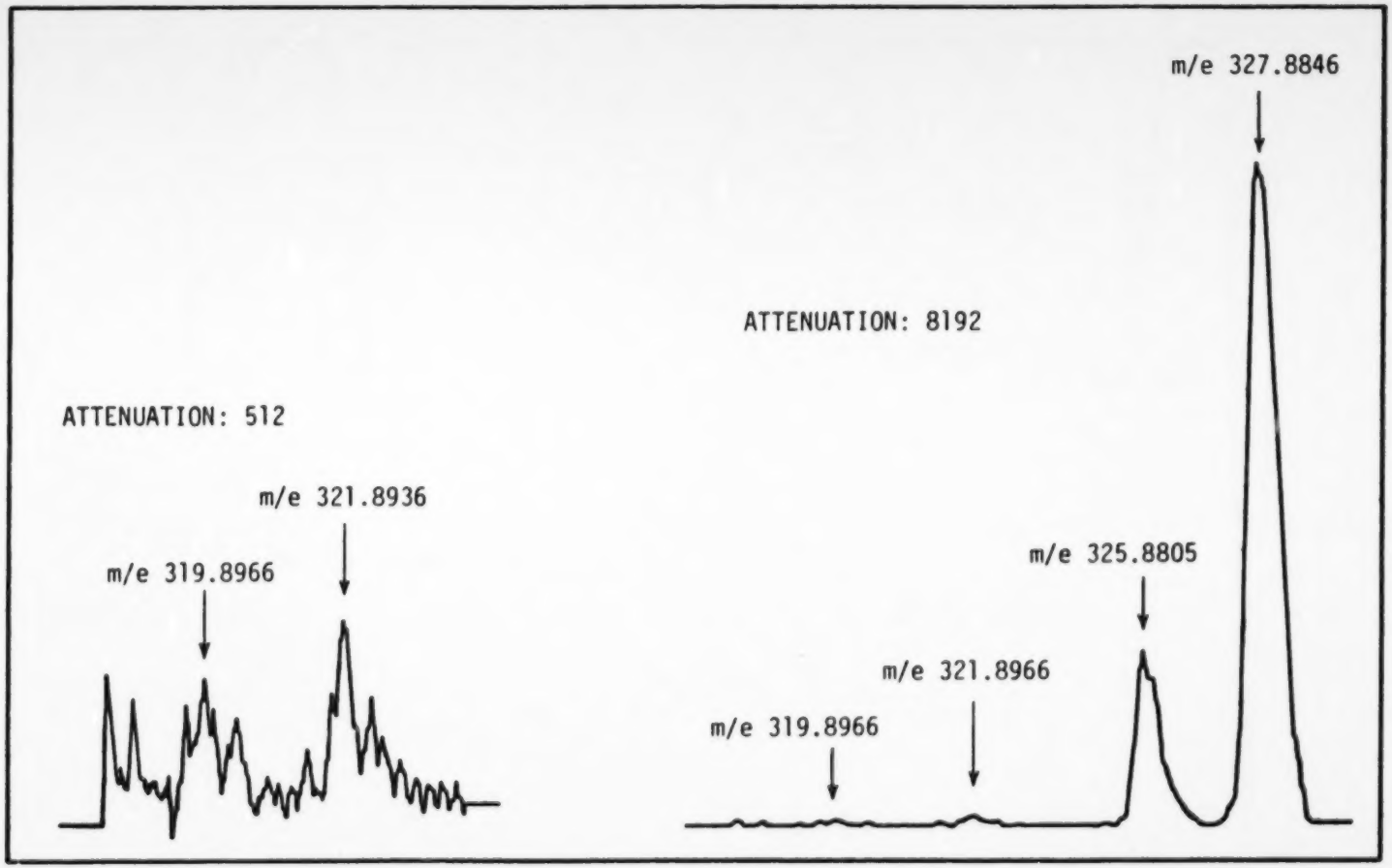

Figure 6. Four-ion mass fragmentogram of sample 12700 obtained with GC-MS-30.

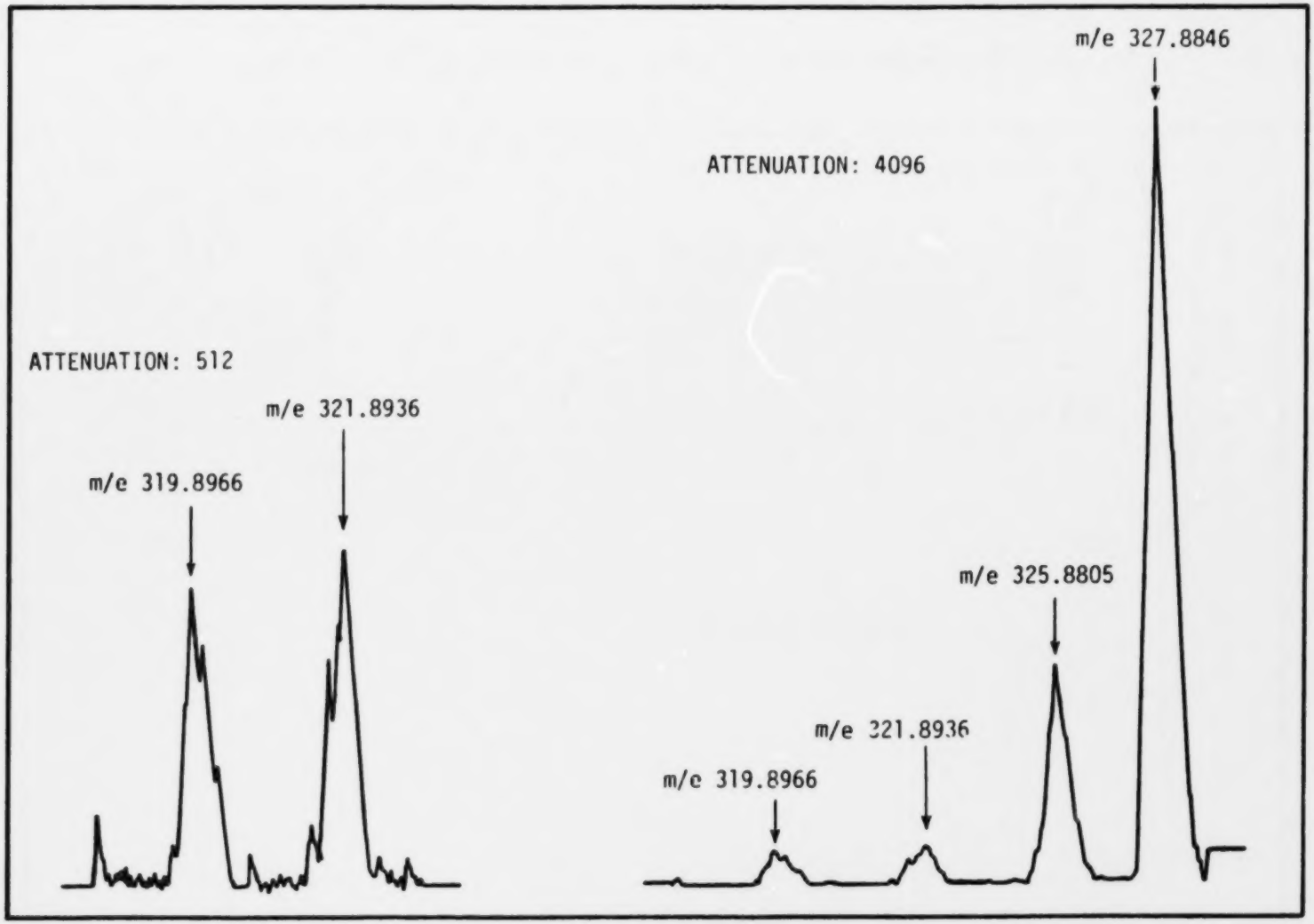

Figure 7. Four-ion mass fragmentogram of sample 5 obtained with GC-MS-30.

TABLE 6. TCDD ISOMER CONTENT OF COLUMN FRACTION SAMPLES SPIKED WITH 2,3,7,8-TCDD

EPA samples[a]	Eluate fraction[b]	Quantity of 2,3,7,8-TCDD added to sample, ng/g	Quantity of 2,3,7,8-TCDD detected in fraction,[c] ng/g	Minimum detectable concentration, ng/g	Recovery, %
C04130	IV	10.42	ND	0.5	
	V		10.62		102
	VI		ND	0.5	
3	V	10.35	597		
	III	50.64	ND	3.0	
	IV		46		
	V		625		
	VI		ND	3.0	
12700	IV	12.14	ND	0.3	
	V		8.4		69
	VI		ND	0.57	
12701	IV	12.84	ND	0.28	
	V		10.12		79
11020	IV	9.86	0.56		6
	V		8.68		88
	VI		ND	0.23	
11024[d]	IV	3.71	0.29		8
	V		1.09		29
	VI		ND	0.08	
11025	IV	6.54	ND	0.14	
	V		5.63		86

[a] See Table 4-1 for description of sample.

[b] Designation of eluate fractions:
- III Third 5-ml aliquot eluted with 20% methylene chloride in hexane.
- IV Fourth 5-ml aliquot eluted with 20% methylene chloride in hexane.
- V First 10-ml aliquot eluted with 50% methylene chloride in hexane.
- VI Last 3-ml aliquot eluted with 50% methylene chloride in hexane.

[c] ND: no 2,3,7,8-TCDD detected in excess of the minimum detectable concentration.

[d] Portion of sample was lost during preparation.

These fractions were analyzed with the GC-QMS in reverse order, beginning with the last fraction and continuing backward until the quantity of TCDD's detected in the several fractions was a reasonably large percentage of that originally added as the spike, or until a fraction was reached that contained no TCDD's. The data in Table 6 show that TCDD's are completely eluted from all samples prior to Fraction VI. In most cases the bulk of the TCDD's appeared in Fraction V, although in samples 11020 and 11024 the TCDD's were detected in Fraction IV.

Table 7 summarizes the total recoveries of the added 2,3,7,8-TCDD spikes achieved by collecting the optimum column chromatography fractions of the various industrial process samples. These recoveries range from 60 to 102 percent, with a mean value of 85 percent.

Except for sample 2, all of the samples processed in this investigation were also spiked with $^{37}Cl_4$-2,3,7,8-TCDD. This compound was added as an internal standard in the analyses with the GC-MS-30 system. The mean recovery of $^{37}Cl_4$-2,3,7,8-TCDD for the samples analyzed herein was 74 percent with a standard deviation of 16.8 percent. The recovery data are shown in Table 8.

Confirmation of TCDD's in Sample 2

Measurements in which m/e 320 and m/e 322 were monitored by the low-resolution GC-QMS system indicated that sample 2 contained approximately 40 µg TCDD's per gram of sample. The report of this high level of TCDD's prompted considerable concern both at EPA and state regulatory organizations.

This finding was also controversial because an earlier examination of this sample in an EPA laboratory had yielded no indication of the presence of TCDD's. It was obviously important, therefore, to more definitively confirm the initial Wright State analyses of sample 2; this was done by a procedure essentially the same as that which is described as the final method (Section 3).

The sample was extracted, and the extract was subjected to liquid chromatography preparation. As mentioned earlier, the fraction of sample 2 that was eluted from the alumina column with 20 percent methylene chloride in hexane was determined to contain the bulk of the TCDD's. Accordingly, this fraction was analyzed for TCDD's by the GC-MS-30 system operated in the dual-ion monitoring mode (m/e 319.8966 and 321.8936 were monitored). The resolution of the MS-30 mass spectrometer was adjusted to 1:12,500 for this measurement.

The dual-ion step-scan mass fragmentogram obtained with this sample extract is shown in Figure 8 and corresponding data obtained with an authentic 2,3,7,8-TCDD standard are shown in Figure 9. For EPA sample 2,

TABLE 7. RECOVERIES OF 2,3,7,8-TCDD-SPIKED SAMPLES FOLLOWING ALUMINA COLUMN CHROMATOGRAPHY

EPA sample no.	Quantity of 2,3,7,8-TCDD added, ng/g (ppb)	Quantity of 2,3,7,8-TCDD detected, ng/g (ppb)	Recovery, %
C04130	10.4	10.6	102
4	12.0	8.4	70
5	12.2	11.0	90
6	10.4	9.7	93
12700	12.1	8.4	69
12701	12.8	10.1	79
11020	9.9	9.24	94
11024	3.7	1.38	37[a]
11025	6.5	5.6	86

[a] Portion of sample lost during preparation.

TABLE 8. RESULTS OF GC-MS-30 ANALYSES OF SAMPLES SPIKED WITH $^{37}Cl_4$-2,3,7,8-TCDD

EPA sample no.	WSU sample no.	Quantity of ^{37}Cl-2,3,7,8-TCDD added, ng/g (ppb)	Quantity of ^{37}Cl-2,3,7,8-TCDD detected, ng/g (ppb)	Recovery, %
C04130	B-001C	1.11	0.78	70
C04131	B-002A	0.93	0.91	98
C04132	B-003A	0.96	0.61	64
5	B-006A	1.21	0.48	40
6	B-007A	1.09	0.67	61
4	B-008A	1.09	0.75	69
12700	B-009E	1.23	1.06	86
12701	B-010E	1.29	1.14	88
11020	B-012F	1.19	0.93	78
11025	B-017B	0.67	0.58	86

[a] Data for samples 2 and 3 are not included because the ratio technique could not be used with samples containing high levels of TCDD. Sample 11024 is also omitted because the extract was not clean enough for analysis by GC-MS-30.

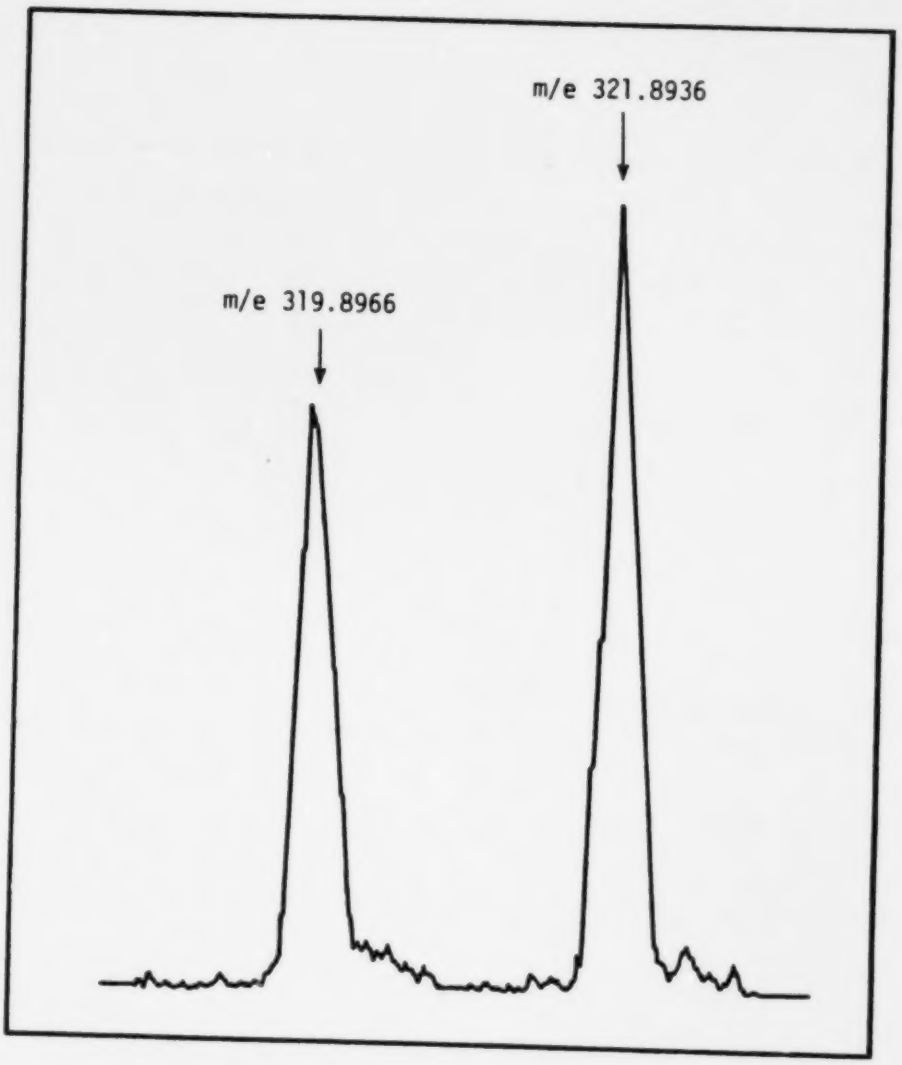

Figure 8. Dual-ion mass fragmentogram of sample 2 obtained with GC-MS-30, mass resolution 1:12,500.

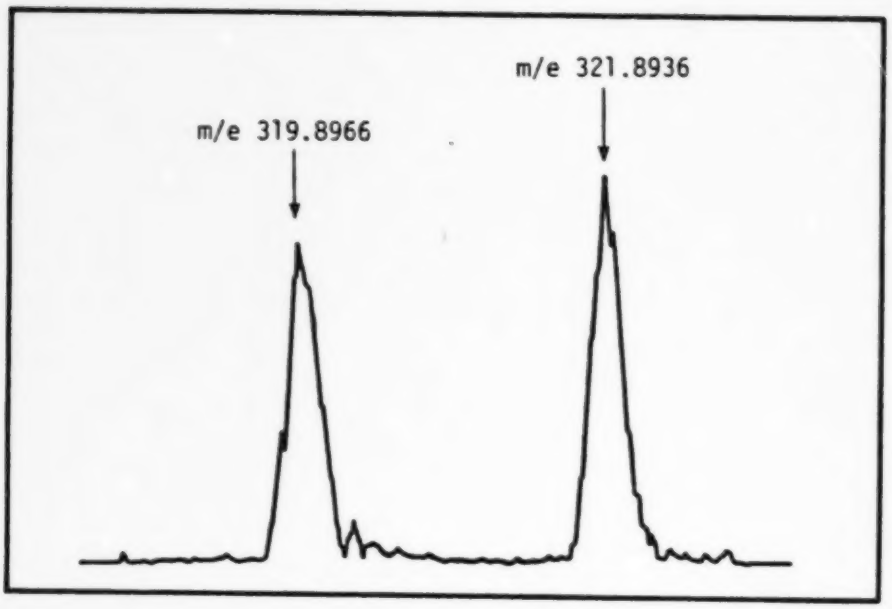

Figure 9. Dual-ion mass fragmentogram of 150 pg of 2,3,7,8-TCDD standard obtained with GC-MS-30, mass resolution 1:12,500.

the ratio of m/e 319.8966 to m/e 321.8936 in the mass fragmentogram is 0.79, while that for the 2,3,7,8-TCDD standard is 0.84. Both of these values agree well with the theoretically predicted ratio of these two peaks, 0.77, which is calculated on the basis of the relative abundance of ^{35}Cl and ^{37}Cl isotopes.

Further confirmation that the unknown component in sample 2 is indeed a quantity of TCDD isomers is provided by the observation that the GC retention time of the unknown component was identical to that of the 2,3,7,8-TCDD standard. This criterion is applied in all determinations of TCDD's in Wright State's Brehm Laboratory.

The mass spectrometric resolution achieved in this program with the MS-30 Mass Spectrometer can be demonstrated experimentally by using the specialized step-scan circuitry developed by Wright State. The practical method of demonstrating the resolution is to obtain a narrow mass scan for a sample consisting of TCDD's in a mixture of other compounds that yield mass spectral ions whose mass is very close to that of TCDD's. In earlier studies we utilized a mixture of 2,3,7,8-TCDD, PCB's such as Aroclor 1254, and DDE* for this purpose. The latter compounds yield mass spectral peaks that are very near the mass of the TCDD's major ion (Aroclor 1254 m/e 321.8679, DDE m/e 321.9290, 2,3,7,8-TCDD m/e 321.8936).

In order to obtain ions of approximately equal intensity from all these compounds, however, the quantities of PCB and DDE must be quite large relative to the quantity of TCDD's. Figure 10 shows a typical mass fragmentogram obtained during this investigation in analyses of two mixtures of 2,3,7,8-TCDD and DDE and a mixture of Aroclor 1254, 2,3,7,8-TCDD, and DDE. On the basis of the data shown in Figure 10, the dynamic resolution of the mass spectrometer is calculated to be 14,000 with 20 percent valley definition.

The data on sample 2 which were described above were based on monitoring only m/e 320 and m/e 322 in the mass spectrum of TCDD's. Our earlier experience had shown that the low levels of TCDD's that are usually found in environmental samples (low ppt) permit monitoring of no more than four mass peaks for a single sample injection, even with the sophisticated step-scan techniques developed in Brehm Laboratory. In this instance, however, the level of TCDD's (40 ppm) in sample 2 was very high and it was feasible to obtain an actual mass spectral scan as this component of the sample eluted from the gas chromatograph.

Therefore the MS-30 Mass Spectrometer was set up in the normal magnetic scanning mode, and an aliquot of the extract of sample 2 was injected into the GC. At the appropriate retention time, the mass spectrum of the eluted component was scanned. Before this, we obtained similar mass spectra of a solution containing 10 ng of authentic 2,3,7,8-TCDD standard and of a solvent blank (benzene). The instrumental parameters applicable to the scans are as follows:

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* As previously noted, DDE is a degradation product of the pesticide DDT.

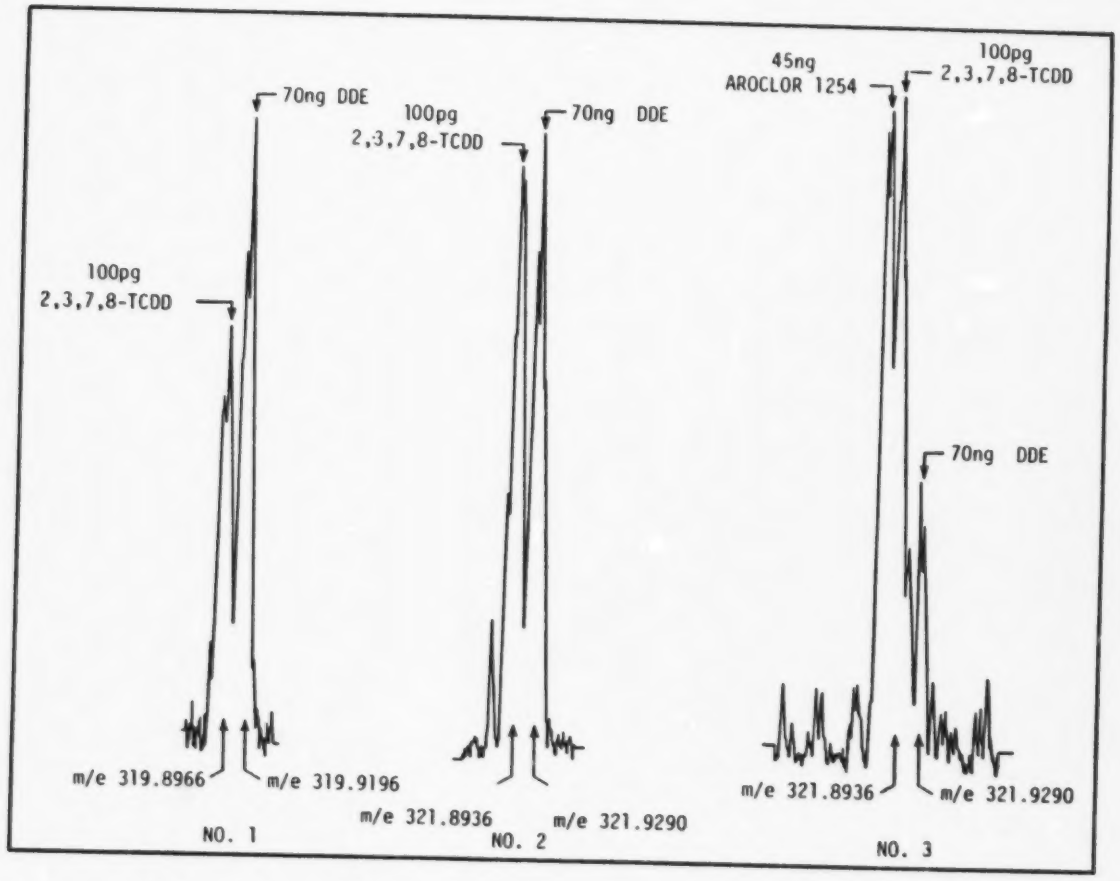

Figure 10. Mass fragmentograms using GC-MS-30 of mixtures of 2,3,7,8-TCDD with other chlorinated compounds.

Scan rate:	10 sec/decade, beginning 190 sec. after sample injection
Mass range of scan:	m/e 130 to m/e 350
Mass resolution:	1:1000
GC retention time for TCDD:	195 sec.
Other parameters:	Same as described in Section 3

The relative intensities of the more prominent mass spectral peaks recorded in these runs are listed in Table 9. The mass spectra obtained for the 2,3,7,8-TCDD standard and for the extract of sample 2 are shown in Figures 11 and 12. These spectra obviously agree quite well. There is no doubt that the unknown component in sample 2 is a TCDD isomer and that it is present in a high concentration. Apparently some components of the extract of sample 2, other than the TCDD's, also contribute to m/e 194, 257, and 259, but these are not of concern here.

TABLE 9. RELATIVE INTENSITIES OF MAJOR IONS OBSERVED IN MASS SPECTRAL SCANS

m/e	10 ng 2,3,7,8-TCDD standard	Solvent blank	10 µl of EPA sample 2 extract (out of 2000 µl total)
326	10	0	12
324	50	0	48
322	100	0	100
320	80	0	80
318	30	0	25
259	23	0	47
257	34	0	48
194	18	0	30
161	21	4	25
160	17	4	20

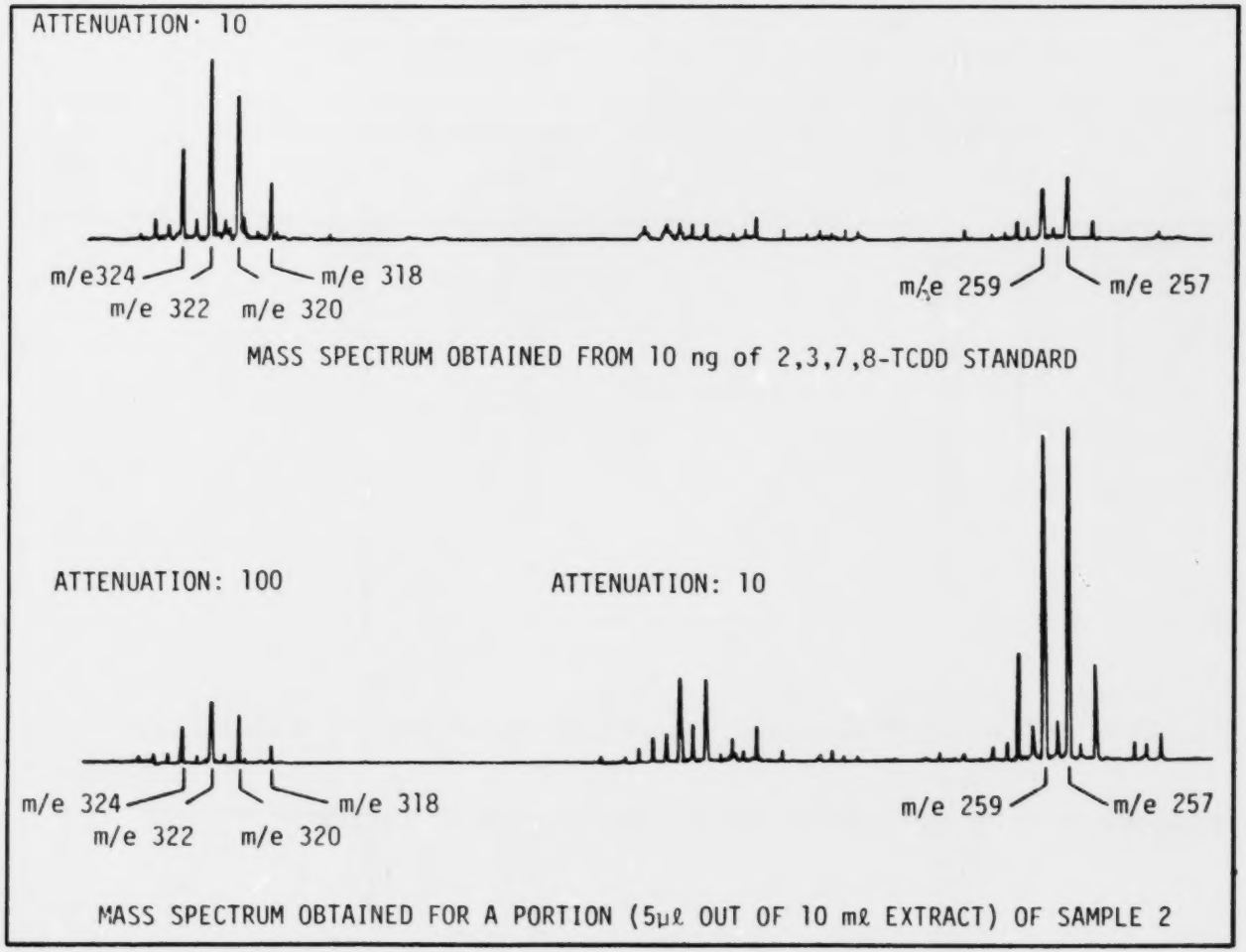

Figure 11. Mass spectra from scans of 2,3,7,8-TCDD standard and sample 2 (mass range m/e 330 to m/e 250).

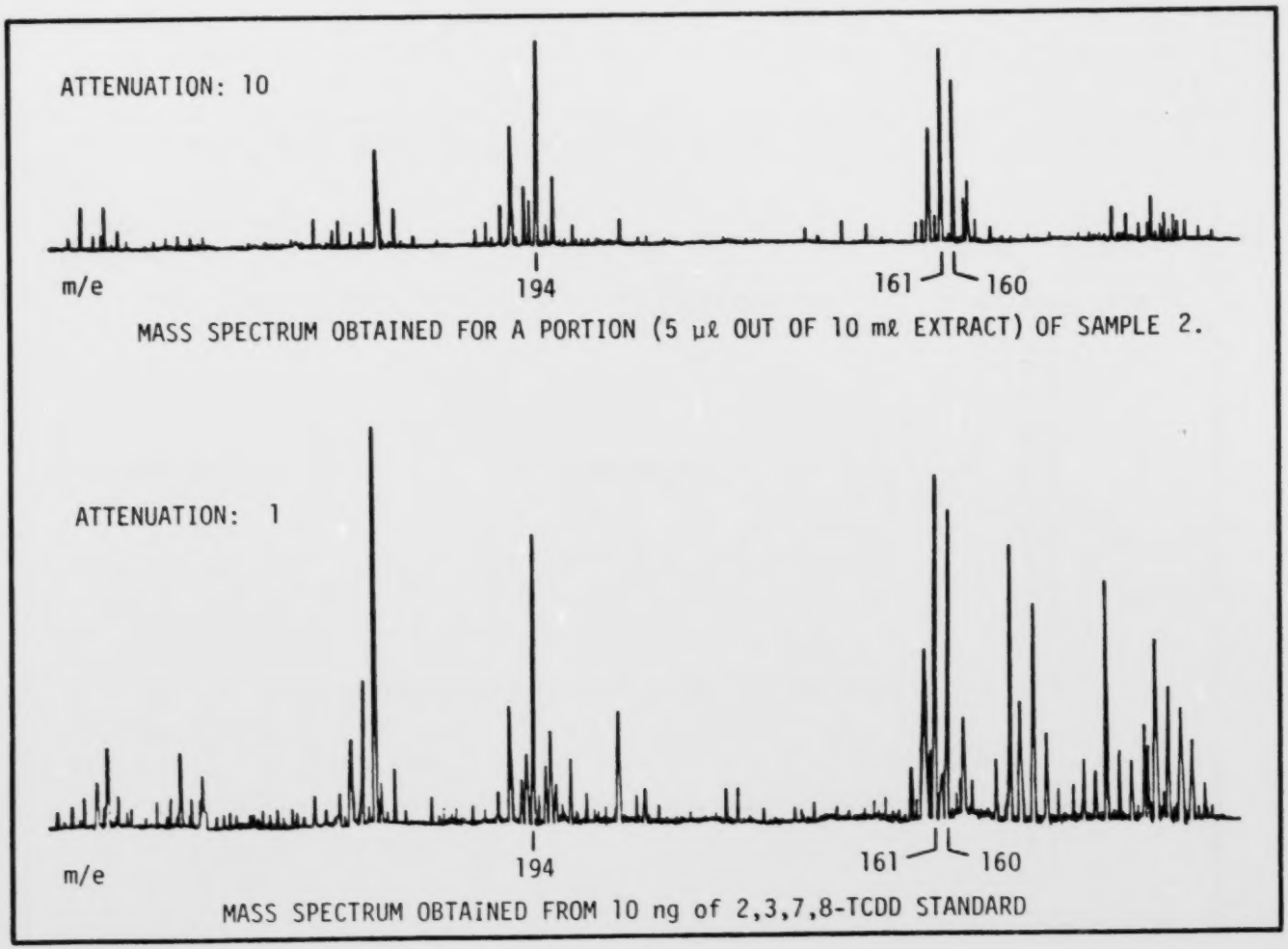

Figure 12. Mass spectra from scans of 2,3,7,8-TCDD standard and sample 2 (mass range m/e 250 to m/e 150).

SECTION 5

CONCLUSIONS AND RECOMMENDATIONS

As a means of assessing the levels of the extremely toxic TCDD's in process streams, wastes, and sediments from the manufacture of chemicals, a method was developed that proved to be applicable to about 70 percent of the industrial waste sample types examined in this study. These sample types are typical of those that would be collected in a routine chemical plant survey.

The analytical methodology implemented in this study is summarized in the following five principal steps:

1. Preparation of a spiked and nonspiked aliquot of each sample in liquid extractable form (organic phase).

2. A sample clean-up procedure that includes acid and base washes to remove the bulk of the sample matrix.

3. An additional sample separation step using liquid chromatography.

4. Screening of samples for detectable levels of TCDD's with a low-resolution GC-QMS system. This step is repeated with a spiked sample if positive levels of TCDD's are detected.

5. Confirmation and quantification of the level of TCDD's by analysis of the samples with a high-resolution GC-MS-30 system.

There are four major advantages with the implementation of this method:

1. The procedure offers a relatively rapid method for qualitative screening of a wide variety of materials for possible contamination by TCDD's, through the use of low-resolution mass spectrometry (GC-QMS showed a MDC of 1 ppb or less in 50 percent of the samples).

2. Only samples in which the initial screening shows TCDD's need be confirmed by use of GC with high-resolution mass spectrometry (minimum resolution 1:10,000).

3. Analysis by high-resolution mass spectrometry yields extremely high sensitivity as well as specificity. The need for both is indicated by the finding of minimum detectable concentrations below 100 ppt in more than half the samples tested.

4. The method warrants a high level of confidence owing to the use of an internal standard and application of the four-ion monitoring technique. Recovery of $^{37}Cl_4$-2,3,7,8-TCDD from spiked samples indicates a recovery range of 40 to 98 percent for the method. Further, by a procedure in which the quantity of native-TCDD's detected is proportionately related to the quantity of $^{37}Cl_4$-2,3,7,8-TCDD added, the data may be automatically corrected for recovery.

Although the procedures outlined here are acceptable for analysis of many industrial process samples, they are not applicable to all sample types. Among those examined in this study, the samples that could not be suitably analyzed are of two types. First are those of biological origin, primarily wood and woodlike products. It is probable that for such samples an acid digestion step is needed to effectively destroy cellular walls and release any residue of TCDD's. Earlier work at Brehm Laboratory on wood and other biological materials confirms the effectiveness of such an approach.

The other type of sample not amenable to the method is more difficult to characterize. Samples of this type formed emulsions in the preparation phase that could not be resolved. Use of several common emulsion-breaking techniques such as addition of excess solvent, did not alleviate this problem. Unfortunately, owing to the small number of samples of this type, no further information was obtained. Additional work on such samples would be desirable.

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APPENDIX A
BASIC PRINCIPLES OF GAS CHROMATOGRAPHY, MASS SPECTROMETRY, AND COMBINED SYSTEMS

GAS CHROMATOGRAPHY (GC)

Gas chromatography is a special form of chromatography that is used to separate the components of chemical mixtures. Several excellent references describe the technique in detail (Dal Nogare and Juvet 1962; Littlewood 1970; Jones 1970; Ambrose 1971). In gas chromatography the mobile phase is a gas and the stationary phase is either a liquid or a solid, hence the terms gas-liquid chromatography and gas-solid chromatography. Gas-liquid chromatography entails the use of a separation device, which is a column containing the liquid phase (typically a high-boiling organic silicone polymer) distributed on a highly inert solid support. Figure A1 depicts a typical gas chromatograph.

The column is maintained in an oven, in which the temperature can be controlled precisely; through the column is passed an inert, high-purity gas (e.g., helium), called the carrier gas. The carrier gas is the mobile phase and the organic silicone polymer is the liquid phase. Typically, the samples are introduced into the column in 0.1 to 10 µl amounts with a microsyringe through an injection port, which is a heated (100° to 250°C) inlet system equipped with a silicone septum. The sample is vaporized immediately upon injection, and The inert carrier gas passing through the injection port sweeps the volatilized, injected sample out of the injection port and into the gas chromatographic column. The volatilized constituents of the sample migrate through the column at varying rates because of variations in the physical and chemical properties of each component, such as boiling point, absorptivity, and solubility. The components are thus separated and emerge (elute) from the column at different times. In some samples the components are highly similar and are not effectively separated or may necessitate the use of extraordinary chromatographic procedures. More commonly, however, the components of a chemical mixture can readily be separated by fairly simple gas chromatographic techniques.

As each separated component elutes from the gas chromatographic column, it is detected by one or more of several types of detectors. Among the widely used detectors are flame ionization, thermal conductivity, and electron capture detectors. Other, more specific, types of detectors are also used in conjunction with gas chromatography; in particular, the mass spectrometer has been used extensively. A discussion of the principles of mass spectrometry follows.

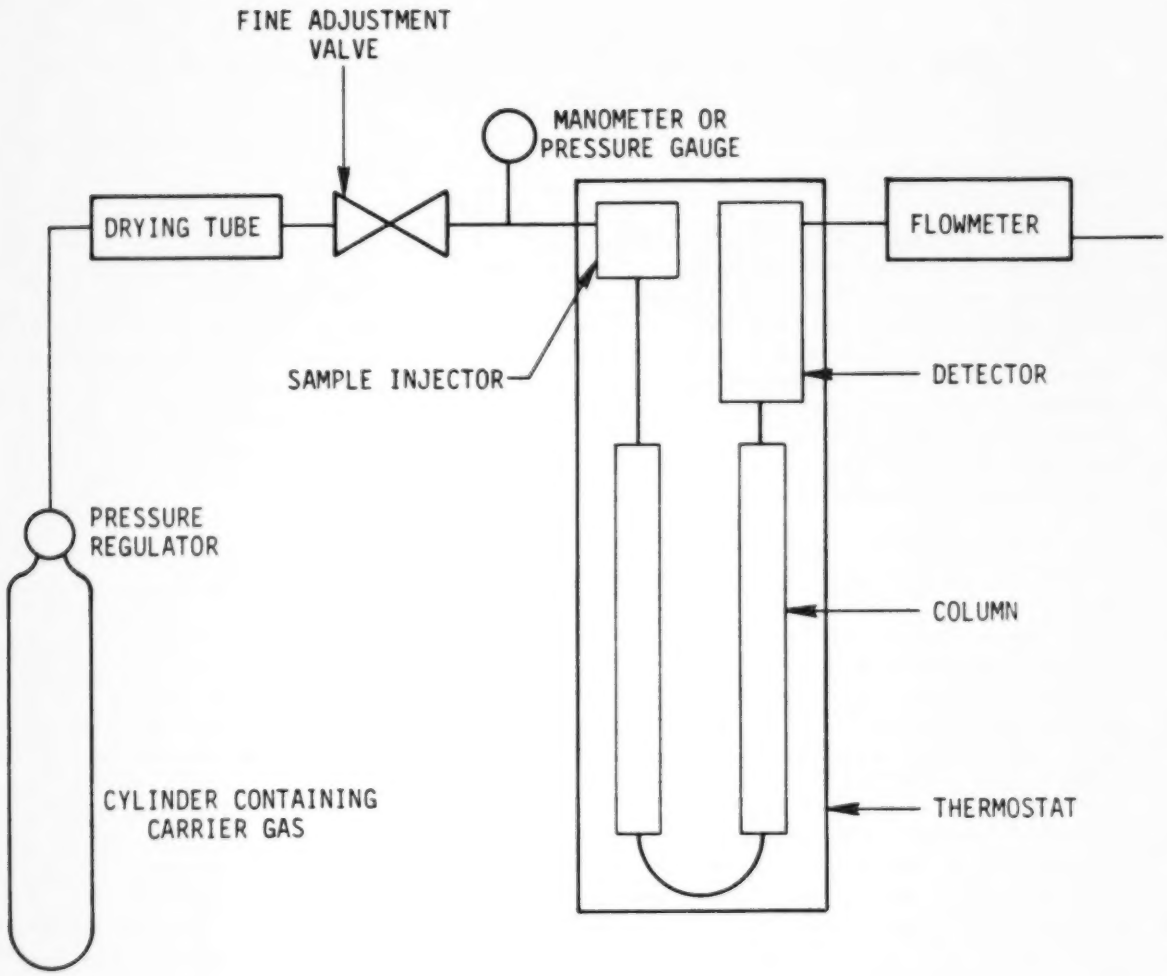

Figure A1. Apparatus for gas chromatography

MASS SPECTROMETRY (MS)

Mass spectrometry is described in detail in several references (Beynon 1960; McLafferty (ed.) 1963; Kiser 1965; Roboz 1968; McFadden 1973). Figure A2 is a schematic diagram of a typical mass spectrometer; the principal components of such a system are (1) an inlet system (2) an ion source, (3) an accelerating system, (4) an analyzer system, (5) a detector, and (6) a data acquisition system. The functions of these components are described briefly.

The inlet system is the means of introducing the sample into the ion source of the mass spectrometer. Inlet devices in common use include heated direct insertion probes and heated gas inlet systems (batch inlets), which are coupled to the mass spectrometer through a restricted fixed or variable orifice, often called a "leak." In recent years the gas chromatograph has been used often to introduce the sample and is coupled to the mass spectrometer--hence the term "coupled GC-MS."

Because the ion source, the accelerating lens system, the mass analyzer, and the detector of the mass spectrometer are all maintained under vacuum by a pumping system, the inlet system must admit the sample (and the carrier gas of a gas chromatograph) into the spectrometer at such a rate that the pumping system maintains the specified internal operating pressure of the instrument.

The ion source (shown schematically in Figure A3) is typically maintained at pressures of 10^{-3}mm and lower (10^{-6}mm) and at temperatures of 100° to 250°C. The source is the region in which ions are generated from the volatile sample molecules admitted through the inlet system. The ionization of molecules in the gas phase is effected by bombarding them with electrons emitted from a hot metal wire or ribbon (the filament) and drawn through a set of slits for collection at an anode or electron trap. The energy of the electrons is controlled by the potential difference between the filament and the trap. As these energetic electrons either strike or pass close to the sample molecules, ionization occurs, producing a molecular ion that usually is fragmented further to yield other ions of smaller mass. The ion source produces both positively charged and negatively charged ions, and many mass spectrometers in use today are designed to detect both types.

The ions produced are electrically forced out of the ion source and into the accelerating lens system, which generally imparts several kilovolts of energy to the ions, which then enter the mass analyzer section.

The purpose of the mass spectrometer analyzer is to separate the ions according to their mass:charge ratios. Various types of analyzer systems are in use today, and the type of analyzer usually provides the descriptive name for each mass spectrometer system. Thus there are, for example, quadrupole mass spectrometers, single-focusing magnetic deflection mass spectrometers, time-of-flight mass spectrometers, and double-focusing mass spectrometers. Each of these systems is characterized by a distinct mode of ion separation, and each provides different capabilities.

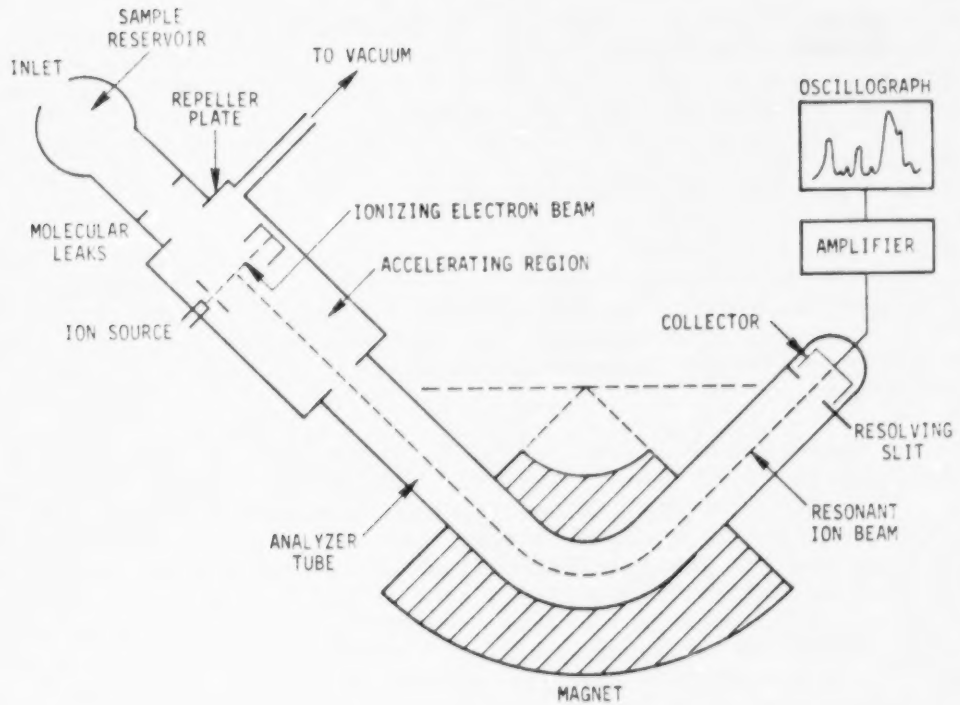

Figure A2. Schematic diagram of a Nier 60° sector mass spectrometer.

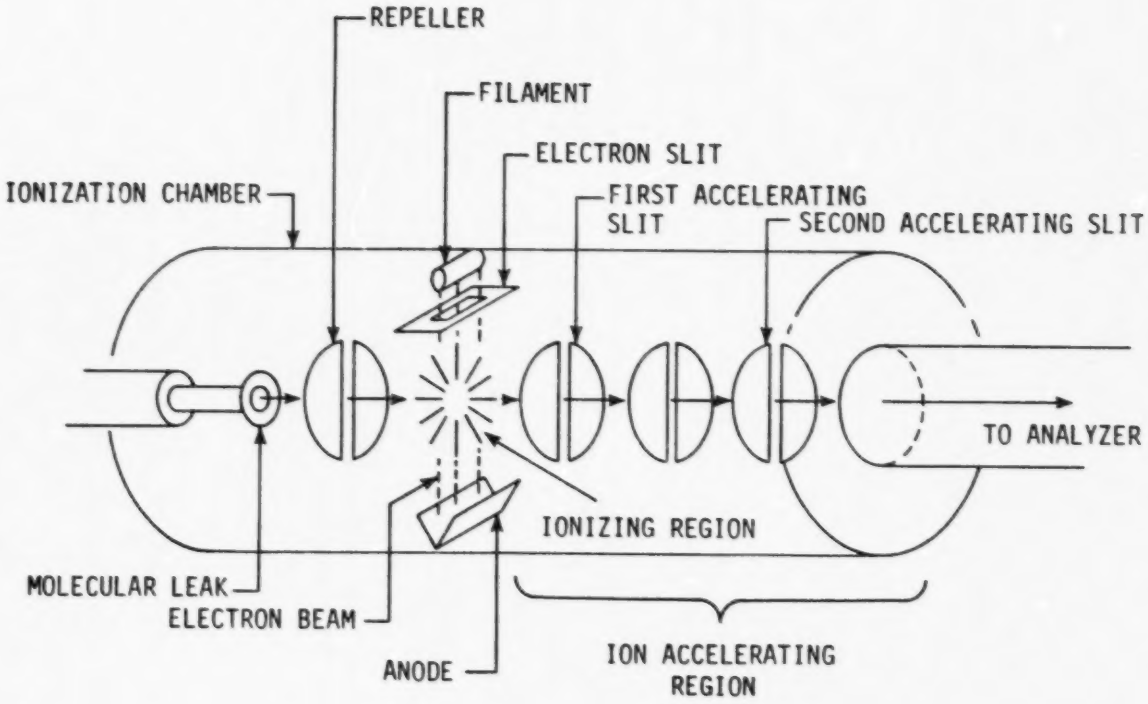

Figure A3. Electron-impact ion source and ion accelerating system.
Source: Merritt and Dean 1974.

The ability of a mass spectrometer to effect a separation of adjacent mass peaks (that is, to resolve these peaks) depends upon the analyzer. Resolution is defined by the equation, $R = M/\Delta M$, where M is the mass of the first peak in a doublet and ΔM is the difference in the masses of the two peaks. An increase in the value of R (denoting an increase in resolution) indicates an increase in the ability to distinguish between very nearly identical masses. Of the several mass spectrometers mentioned, the double-focusing type affords the greatest mass spectral resolution, sometimes exceeding 100,000. At this degree of resolution, masses appearing at m/e 99,999 and m/e 100,000 would be distinguishable. An instrument capable of such high resolution is of course very complex and expensive and thus would be used only when such high resolution is mandatory for effective analysis. In contrast, a quadrupole mass spectrometer is much simpler to operate and less expensive but can provide only low resolution ($m/\Delta m$ = 500 to 1000 typically).

Detection of the ions that have been separated is accomplished most often by use of an electron multiplier, of which, again, various types are in use. An electron multiplier produces current amplification of 10^3 to 10^8 with very low noise level and with negligible time constant or signal broadening. The amplified analog signal resulting from the ion impacting on the electron multiplier is finally routed to one of several possible data acquisition devices; among those often used are the ocillographic recorder, the analog recorder, a pulse counting device, or the digital computer.

The data from a mass spectrometer consist, in the analog format, of a spectrum of peaks (the mass spectrum). The position of each peak on the horizontal axis of a graphic display indicates its m/e ratio whereas the amplitude of each peak indicates the number of ions (or abundance) of that m/e. The data may also be displayed digitally in tabular form.

If more than one compound enters the mass spectrometer at a given time, then the masses detected are generally attributable to any or all of the compounds. Because it is difficult, and sometimes impossible, to interpret the mass spectra obtained for mixtures of organic compounds, there is great advantage in admitting the compounds separately. Thus a gas chromatograph is used to introduce the separated components of a mixture sequentially into the mass spectrometer. Following is a simplified description of a coupled GC-MS system.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC-MS) SYSTEMS

In considering the coupling of the gas chromatograph to a mass spectrometer, one should recall that the source, analyzer, and detector of the spectrometer are all typically maintained at pressures below 10^{-5}mm. Therefore, unless the mass spectrometer is equipped with a very high-capacity pumping system, the gaseous effluent from a gas chromatographic column cannot be admitted directly to the mass spectrometer source because this would increase the pressure to a level that would prevent satisfactory operation. Therefore, coupling is generally achieved by use of an inter-

mediate device to reduce the rate of flow of the sample and carrier gas stream. For this purpose several types of devices (called "separators") are used to achieve partial separation of the carrier gas (typically helium) from the gaseous sample molecules. Among these devices are (1) a porous barrier or effluent splitter, (2) a jet/orifice separator, and (3) a molecular separator that includes a permeable membrane. Some gas chromatograph/mass spectrometer systems feature a direct coupling of the gas chromatograph with the mass spectrometer by means of a very high capacity pumping system.

A system that couples a chromatograph with a mass spectrometer is a very powerful analytical tool, the only system that can provide definitive analysis of complex chemical mixtures. The separation capabilities of the gas chromatograph are complimented by the inherent specificity and sensitivity of the mass spectrometer. During analysis of a complex mixture, the components are separated gas chromatographically, each eluted component then passes through the interface (separator) and into the mass spectrometer, which provides and records a mass spectrum. Typically, the analysis of a mixture could yield several hundred mass spectra, each containing 100 to 200 mass peaks. Therefore, the computer is an ideal means of acquiring the mass spectra, reducing the data (converting the acquired data to actual mass spectra by comparison with calibrated reference files), and displaying the data. The minicomputer is an essential component of a modern GC/MS system because the analyses generate such sizable quantities of data. Use of a minicomputer can afford other advantages; for example, the computer can be programmed to control the mass spectrometer so that it monitors only selected masses typical of the compounds of interest. The computer also can be programmed to allow monitoring of different masses (corresponding to different compounds) at different gas chromatographic retention times.

APPENDIX A

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APPENDIX B

OTHER INSTRUMENTAL METHODS FOR DIOXIN ANALYSIS

Most of the current technology for detection of TCDD's is based on gas chromatography and/or mass spectrometry. However, a variety of other less specific techniques have been used including ultraviolet spectroscopy (Pohland and Yang 1972), electron spin resonance spectroscopy, and low-temperature phosphorescence emission spectroscopy (Baughman 1974). None of these methods provide both the high sensitivity and selectivity needed for analysis of most environmental samples.

A resin sorption technique using XAD-2 resin has achieved a detection limit of 1 ppt for TCDD's in water; because this technique required a large quantity of sample for extraction, however, extension to other types of samples is unlikely (Junk 1976).

Another technique uses PX21 powdered charcoal suspended on shredded polyurethane foam as the sorbant (Huckins, Stalling, and Smith 1978). The TCDD's were eluted from the charcoal column by use of a 50 percent solution of toluene in benzene and finally were detected by electron-capture gas chromatography. To enhance selectivity, an alumina column chromatography step is usually included after elution from the charcoal column. The detection limit of this method ranges from 10 to 100 ppb.

Thin-layer chromatography has also been used for the detection of TCDD's (Williams and Blanchfield 1971). Two-dimensional development with two different solvents is used to increase selectivity. The spot corresponding to 2,3,7,8-TCDD is removed from the plate, extracted with benzene, and detected by electron-capture gas chromatography. This method has achieved a detection limit in the low ppm region.

Steam distillation has also been tried (Storhen 1971), but was suitable only for levels of TCDD's in the range of 1 to 3 ppm and lacked the selectivity needed to avoid interferences.

Recently analytical methods involving chemical ionization mass spectrometry with negative ions have been published. An early communication by Hunt and co-workers (Hunt, Harvey, and Russel 1975) reported a signal-to-noise ratio of 50 from a 2-pg direct-probe insertion sample using oxygen as the reagent gas. A sensitivity 25 times higher than the direct-probe insertion method is reported for electron impact ionization. Hass et al. compare the relative sensitivities of various chemical ionization modes, including those of positive-ion versus negative-ion modes with methane, oxygen, and

mixed methane/oxygen as reagent gases (Hass 1978). Positive-ion chemical ionization affords the greater sensitivity, but does not produce ions indicative of the molecular weight.

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APPENDIX C

LITERATURE REVIEW

This appendix is a compilation of references on dioxin analysis categorized by sample matrix. The categories are given below:

Air	Hexachlorobenzene
Biological tissue	Insecticides
Blood	Milk or cream
Commercial chlorophenols	Plant material
Fats or oils	Soil
Fish and crustaceans	Urine
Flue Gas	Water
Fly ash	Wipe samples
Grain	Wood
Herbicide formulations	

Air

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